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ENVIRONMENTAL RISK FACTORS FOR AUTISM SPECTRUM DISORDERS

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Front cover artist: **James Edwards**. James is a young man who has a diagnosis of Asperger syndrome. He enjoys expressing himself through music and art.

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ABSTRACT

Aims: Two overarching hypotheses were tested in this thesis- first, that the environmental factors studied during pregnancy or the time preceding birth would be associated with a higher risk of autism spectrum disorders (ASD) in the offspring; and second, that these risk factors and/or their magnitude of associations may be different for autism spectrum disorders with and without intellectual disability (ID).

Methods: Studies I-IV were case-control studies nested within a population-based cohort of all children 0 to 17 years old, living in Stockholm County between the years 2001 to 2007 (n=589,114). ASD cases, identified using multisource case-ascertainment, were matched by age and sex to 10 living non-ASD controls. Prospectively collected information on exposures and potential confounders was ascertained by record linkage with relevant registers, and timed to the prenatal period. Exposures included measures of parental socioeconomic status (Study I), migration (Study II), life events (Study III) and parental depression and maternal antidepressant use during pregnancy (Study IV). For Study III, an additional cohort in England (maximum n = 11554) was used to study the risk of offspring ASD in relation to a combined maternal exposure to up to 42 common and rare life events, as well as their perceived impact upon the mother during pregnancy and early life.

Results: In Study I, measures of a lower parental socioeconomic status – specifically, lower household income, and unskilled, manual or unclassified occupations were associated with a higher risk of ASD. The associations were similar in ASD with or without ID. In Study II, maternal migration had divergent relationships with ASD with and without ID- showing heightened risks for ASD with ID and reduced ones for ASD without ID. This study found that associations of migration with autism varied by the geographical region of origin of the mother, by the human development of the region of origin, and the timing of migration in relation to pregnancy. In Study III, no evidence for a relationship between stressful life events during pregnancy and a heightened risk of ASD was found, using data from the two population-based studies in Sweden and England respectively. In Study IV, a higher risk of ASD was associated with a prenatal history of maternal depression, but did not appear to be associated with paternal depression. In a smaller sample, when maternal antidepressant use was simultaneously studied, the associations of maternal depression with ASD appeared to be confined to the group of women who reported taking antidepressants during pregnancy. The associations were higher for ASD without ID, and were not observed for ASD with ID.

Conclusion: In three of the four studies there was evidence of a relationship between the prenatal factors studied and a higher risk of autism spectrum disorders. In two studies, the timing of the event (migration, antidepressant use or severe depression during pregnancy) was indicative of pregnancy related exposures, highlighting the importance of considering environmental factors acting *in utero* in the pathways to autism. The marked differences in risks for autism with and without intellectual disability with exposures in two studies highlight the value of studying these categories separately, since they may have different determinants.

LIST OF PUBLICATIONS

- I. RAI D, Lewis G, Lundberg M, Araya R, Svensson A, Dalman C, Carpenter P, Magnusson C. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study.
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- II. Magnusson C, RAI D, Goodman A, Lundberg M, Idring S, Svensson A, Koupil I, Serlachius E, Dalman C. Migration and autism spectrum disorder: population-based study.
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- III. RAI D, Golding J, Magnusson C, Steer C, Lewis G, Dalman C. Prenatal and early life exposure to stressful life events and risk of autism spectrum disorders: population-based studies in Sweden and England.
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- IV. RAI D, Lee BK, Dalman C, Golding J, Lewis G, Magnusson C. Parental depression, maternal antidepressant use during pregnancy and risk of autism spectrum disorders: population-based study.
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CONTENTS

1	Background.....	1
1.1	An introduction to autism spectrum disorders	1
1.2	Classification and subtypes	1
1.3	Reports of rising prevalence and public health implications.....	3
1.4	The aetiology of autism spectrum disorders	3
1.4.1	Genetic and environmental influences	3
1.4.2	Why study environmental factors?	5
1.4.3	A conceptual framework	6
1.4.4	Parental socioeconomic status.....	7
1.4.5	Parental migration	8
1.4.6	Stressful life events.....	8
1.4.7	Parental depression and maternal antidepressant use	9
2	Aims	11
2.1	Overall hypothesis	11
2.2	Specific aims	11
2.2.1	Study I.....	11
2.2.2	Study II.....	11
2.2.3	Study III	11
2.2.4	Study IV	11
3	Materials and Methods	12
3.1	Record linkage design	12
3.2	Study population: The Stockholm Youth Cohort	12
3.2.1	Register linkages in the Stockholm Youth Cohort	12
3.2.2	Autism spectrum disorders in the SYC.....	14
3.2.3	Nested case-control design.....	15
3.2.4	Exposure variables.....	16
3.3	The Avon Longitudinal Study of Parents and Children (Study III)	20
3.3.1	Autism spectrum disorders in ALSPAC	20
3.3.2	Life event measures in ALSPAC	20
3.4	Statistical analysis.....	21
3.5	Ethical approval	22
4	Results.....	23
4.1	Study I.....	23
4.2	Study II.....	25
4.3	Study III	28
4.4	Study IV	31
5	Discussion.....	34
5.1	Main findings in the context of previous literature.....	34
5.1.1	Socioeconomic patterning of autism may be similar to other developmental disorders	34
5.1.2	Migration and autism- relationships beyond geographic conceptualizations.....	34
5.1.3	No evidence of a link between acute life events and autism..	35
5.1.4	Depression or antidepressant use during pregnancy?.....	35
5.2	Methodological considerations	35

	5.2.1 Record linkage designs in epidemiological studies.....	35
	5.2.2 Selection bias	36
	5.2.3 Information bias.....	36
	5.2.4 Confounding	38
	5.2.5 Statistical power	38
	5.2.6 External validity	39
5.3	Possible explanatory mechanisms.....	39
	5.3.1 Study I.....	39
	5.3.2 Study II	40
	5.3.3 Study III.....	41
	5.3.4 Study IV	41
6	Conclusions and future directions.....	43
7	Acknowledgements	45
8	References	47

LIST OF ABBREVIATIONS

ALSPAC	Avon Longitudinal Study of Parents and Children
ASD	Autism Spectrum Disorder
ATC	Anatomical Therapeutic Classification of drugs
CI	Confidence Interval
DSM	Diagnostic and Statistical Manual
HFA	High Functioning Autism
HDI	Human Development Index
ICD	International Classification of Diseases
ID	Intellectual Disability
LFA	Low Functioning Autism
MBR	Medical Birth Register
MRI	Monoamine Reuptake Inhibitor
OR	Odds Ratio
PIN	Personal Identity Number
SES	Socioeconomic Status
SSRI	Selective Serotonin Reuptake Inhibitor
SYC	Stockholm Youth Cohort
NPR	National Patient Register
TPR	Total Population Register
UNDP	United Nations Development Programme
WHO	World Health Organization

1 BACKGROUND

1.1 AN INTRODUCTION TO AUTISM SPECTRUM DISORDERS

Seventy years ago, the eminent child psychiatrist Leo Kanner published his seminal account of 11 children with an ‘autistic disturbance of affective contact’, who had features of extreme aloneness (the reason he used the term autism), an interest in objects rather than people, rigidity, repetitiousness, and a powerful desire for sameness amongst other symptoms (Kanner, 1943).

One year later, Hans Asperger, an Austrian paediatrician published his own account of children with ‘autistic psychopathy’ in German (Asperger, 1944). His work would remain largely unknown to the English speaking world until his observations were summarized by Lorna Wing (Wing, 1981) as Asperger’s syndrome, and later translated into English by Uta Frith (Frith, 1991).

Wing pointed out that there were many similarities in the clinical descriptions of the children described by Kanner and Asperger, and based on the findings of a study conducted with Judith Gould, proposed a triad of impairments (Wing and Gould, 1979) consisting of an:

...absence or impairment of two-way social interaction; absence or impairment of comprehension and use of language, non-verbal as well as verbal; and absence or impairment of true, flexible imaginative activities, with the substitution of a narrow range of repetitive, stereotyped pursuits (Wing, 1981)

The concept of the autism ‘spectrum’ was thus born, encapsulating the idea that each aspect of this triad of impairments could vary in severity, and be present in individuals with all levels of intellectual functioning. This triad was widely adopted and operationalized into diagnostic criteria which are still in use (Rutter and Schopler, 1987; Rutter and Schopler, 1992).

1.2 CLASSIFICATION AND SUBTYPES

The characteristic features of autism spectrum disorders are considered to include impairments in reciprocal social interaction, communication, and restricted patterns of interests and stereotyped behaviours. Although the term ‘autism spectrum disorder’ has come into general parlance in recent years, this term currently does not exist in either of the main classification systems. The various conditions considered to comprise the ‘autism spectrum’ are included in the category of pervasive developmental disorders in both the Diagnostic and Statistical Manual, currently DSM-IV (American Psychiatric Association, 2000), and the International Classification of Diseases, currently ICD-10 (World Health Organization, 1993).

For instance, within the ICD-10 these include Infantile autism, Asperger syndrome, and Pervasive developmental disorder, unspecified (**See Box 1**). The nosological validity of

these specific categories remains a topic of debate, and the boundaries between the various subclasses have been found to be unreliable even when rigorously applied, using strict operational definitions, at centres of research excellence (Lord *et al.* 2011).

Box 1 ICD-10 Criteria for Pervasive developmental disorders which are now commonly considered as Autism spectrum disorders

F84 Pervasive developmental disorders *A group of disorders characterized by qualitative abnormalities in reciprocal social interactions and in patterns of communication, and by a restricted, stereotyped, repetitive repertoire of interests and activities. These qualitative abnormalities are a pervasive feature of the individual's functioning in all situations.*

F84.0 Childhood autism *A type of pervasive developmental disorder that is defined by: (a) the presence of abnormal or impaired development that is manifest before the age of three years, and (b) the characteristic type of abnormal functioning in all the three areas of psychopathology: reciprocal social interaction, communication, and restricted, stereotyped, repetitive behaviour. In addition to these specific diagnostic features, a range of other nonspecific problems are common, such as phobias, sleeping and eating disturbances, temper tantrums, and (self-directed) aggression.*

F84.1 Atypical autism *A type of pervasive developmental disorder that differs from childhood autism either in age of onset or in failing to fulfil all three sets of diagnostic criteria. This subcategory should be used when there is abnormal and impaired development that is present only after age three years, and a lack of sufficient demonstrable abnormalities in one or two of the three areas of psychopathology required for the diagnosis of autism (namely, reciprocal social interactions, communication, and restricted, stereotyped, repetitive behaviour) in spite of characteristic abnormalities in the other area(s). Atypical autism arises most often in profoundly retarded individuals and in individuals with a severe specific developmental disorder of receptive language.*

F84.5 Asperger syndrome *A disorder of uncertain nosological validity, characterized by the same type of qualitative abnormalities of reciprocal social interaction that typify autism, together with a restricted, stereotyped, repetitive repertoire of interests and activities. It differs from autism primarily in the fact that there is no general delay or retardation in language or in cognitive development. This disorder is often associated with marked clumsiness. There is a strong tendency for the abnormalities to persist into adolescence and adult life. Psychotic episodes occasionally occur in early adult life.*

F84.8 Other pervasive developmental disorders

F84.9 Pervasive developmental disorder, unspecified

Source: *International Classification of Diseases, 10th Edition. Published by the World Health Organization. Electronically available at:*
<http://apps.who.int/classifications/icd10/browse/2010/en#/F80-F89>

There have therefore been proposals to consider these various subtypes under a single autism spectrum disorder category. This possibility is being considered by both of the major classification systems (the ICD and DSM) as they prepare to unveil future editions after revision (American Psychiatric Association, 2011). However, the debate regarding this strategy still continues (Wing *et al.* 2011).

The idea of differentiating between autism according to co-existent intellectual disability (ID) has also been long promoted as a strategy (Bartak and Rutter, 1976), which has practical implications for education and health services, and the management of these conditions (Wing, 1981). More recently, this approach has been supported by both clinical and population-based studies (Lord *et al.* 2011; Szatmari *et al.* 2007; Witwer and Lecavalier, 2008) as an approach that may be more useful for aetiological investigations than the various subcategories of autism in current classification systems.

1.3 REPORTS OF RISING PREVALENCE AND PUBLIC HEALTH IMPLICATIONS

Although considered rare till even two decades ago, autism is now considered an ‘important public health concern’ since the estimated prevalence appears to have risen over 20-fold since the 1980’s (Centers for Disease Control and Prevention, 2012). Many explanations have been cited for this increasing prevalence, such as increasing knowledge and awareness about autism in professionals as well as parents, changes in definitions, and inclusion of relatively milder groups of disorders into the autism spectrum. However, the possibility that these conditions have truly risen in incidence has also been considered, and yet to be ruled out (Fombonne, 2009; Rutter, 2005b).

This rising prevalence also has major implications for costs at all levels, from individuals and families to society. Considering that these disorders present early in life, and are often associated with long term disabilities, their public health impact is high (Ganz, 2007). There are relatively little data quantifying costs, but one recent study estimated that the costs of supporting children with autism were £2.7 billion and £25 billion for adults each year in the UK (Knapp *et al.* 2009). This study estimated a lifetime cost, after discounting, for individuals with ASD and ID as £1.33 Million, and £0.8 million for ASD with ID (Knapp *et al.* 2009).

1.4 THE AETIOLOGY OF AUTISM SPECTRUM DISORDERS

1.4.1 Genetic and environmental influences

The causes of autism spectrum disorders are not well known, despite several decades of research (Gardener *et al.* 2009; Gardener *et al.* 2011; Rutter, 2005a). Before delving further into the role of genetic or environmental factors in this context, it is important to consider some historical aspects which shaped the science as well as public attitude towards autism.

The idea of familial influences on autism was supported in the writings of both Leo Kanner and Hans Asperger. For instance, Asperger considered ‘autistic psychopathy’ to

be genetically transmitted, and noticed similar symptoms in the families, especially fathers of children he described (Wing, 1981).

Similarly, Kanner, in his initial case-series also documented that:

There is a great deal of obsessiveness in the family background. The very detailed diaries and reports, and the frequent remembrance, after several years..., furnish a telling illustration of parental obsessiveness

followed by:

For the most part, parents, grandparents and collaterals are persons strongly preoccupied with abstractions of a scientific, literary or artistic nature and limited in genuine interest in people (Kanner, 1943)

The descriptions above are consistent with a history of autism-like symptoms in the families of children with autism. Kanner thoughtfully ruled out the possibility of attributing the child's condition to be related to 'early parental relations', arguing that children appeared to have these symptoms from birth, and therefore concluded that these individuals had an 'innate' or 'inborn autistic disturbance of affective contact' (Kanner, 1943).

In his follow-up paper in 1949, now with an expanded case sample of 55 children, Kanner still noted the above characteristics in the parents. However, his writing suggested that he now considered that features of autism, particularly the 'aloofness' of the children may have stemmed from a lack of parental warmth:

Most of the patients were exposed from the beginning to parental coldness, obsessiveness, and a mechanical type of attention to material needs only. They were the objects of observation and experiment conducted with an eye on fractional performance rather than with genuine warmth and enjoyment. They were kept neatly in refrigerators which did not defrost. Their withdrawal seems to be an act of turning away from such a situation to seek comfort in solitude (Kanner, 1949)

Thus there appeared to be a shift in his thinking about the aetiology of autism, and the (now widely discredited) theory of 'refrigerator parents' was born, and later propagated vigorously throughout the 1950's and 1960's by several psychoanalysts and child psychologists of the time, most notably by Bruno Bettelheim (Bettelheim, 1967).

Subsequently, a generation of parents suffered the guilt and stigma of somehow being responsible for their child's autism. Thus early in its history, the thinking behind the aetiology of autism moved from 'nature' to 'nurture', but without robust evidence for either, and caused a great deal of distress to a large number of families and parents of children with autism.

The publication of the first twin study of autism in 1977 by Folstein and Rutter marked the beginning of the end of this dark chapter of blaming parents for the aetiology of autism (Folstein and Rutter, 1977). In this study, the authors rigorously examined 21 same sex twin pairs, with at least one twin with infantile autism, and reported a 36% (4

out of 11) concordance rate for monozygotic (MZ) Twins and 0% (none out of 10) concordance for dizygotic (DZ) twin pairs. Within 12 out of 17 twin pairs discordant for autism, evidence was found of a 'biological hazard' that may lead to brain injury, and the authors concluded that brain injury on its own or in combination with a genetic predisposition was likely to be implicated in the causation of autism.

If the above study were published today on an unstudied condition, it could reasonably be concluded that the small sample size did not allow robust conclusions; but that the results would suggest that both environmental and genetic factors could be considered important. However, despite the inherent limitations in the evidence, the above study single-handedly heralded a paradigm shift in the thinking about autism, which henceforth 'became' a genetic disorder. 'Nature' as Asperger and Kanner had initially conceptualized, had once again overtaken 'nurture', which at that time most prominently implicated parenting practices in the aetiology of autism.

As three further twin studies were published in the next two decades, replicating Folstein and Rutter's findings to varying extents (Bailey *et al.* 1995; Ritvo *et al.* 1985; Steffenburg *et al.* 1989), the idea of autism as a highly heritable, genetic disorder was consolidated and widely embraced (Ronald and Hoekstra, 2011), and the controversial parenting theories were cast into oblivion.

This historical background and the role of the initial twin studies in reducing the widespread stigma associated with the parenting theories is important to note. It moved the focus away from the study of other potentially relevant environmental factors in the pathways to autism.

1.4.2 Why study environmental factors?

In one of the classic papers of behavioural genetics, Plomin and Daniels highlighted that by 1987, the role of genetics had become widely accepted in problems that were previously considered environmental:

Ten years ago, in order to redress the imbalance of environmentalism, it was necessary to emphasize the possibility that genetic influence could affect behavioural differences that we observe among individuals...

They went on to highlight that the twin studies, upon which much of the genetic evidence was based, also provided the strongest evidence for environmental influences. Citing Schizophrenia as an example they wrote:

...for schizophrenia, the concordance for first-degree relatives, whose coefficient of genetic relationship is .50, is less than 10%. Identical twins are less than 50% concordant for schizophrenia. Yet schizophrenia is coming to be viewed as a genetic disease. In the rush to find neural causes of schizophrenia, who is now studying the major source of variability - the environment? (Plomin and Daniels, 1987)

Based on the twin studies cited above, autism has come to be known as one of the most heritable conditions known to man but even 35 years after the Rutter and Folstein's first study, and despite major advances in the genetics of autism (Geschwind, 2011; Ronald and Hoekstra, 2011), specific genetic mechanisms are unknown for a majority of cases. The most recent and largest twin study conducted on a well characterized sample urged scientists to consider the importance of environmental factors, reporting that almost 55% of the variance in ASD could be attributed to shared environmental factors (Hallmayer *et al.* 2011).

It is important to state that the premise of this thesis is not at all to question the role of genetics in the aetiology of autism, nor is it to debate what factors - genetic or environmental may be more important in the context of autism. The presumption is that genetic *as well as* environmental factors are probably important, which, like the example of Schizophrenia cited by Plomin and Daniels above, is a fact illustrated within the results of all the twin studies of autism to date (Hallmayer *et al.* 2011; Ronald and Hoekstra, 2011).

Identification of modifiable environmental determinants of autism holds the promise of providing a means to inform public health action and preventative approaches, fulfilling a central aim of epidemiology (Morris, 1955). Furthermore, such factors may explain the rising prevalence of autism. They could also inform efforts towards a better understanding of the complex interactions between genes and the environment in the expression of these disorders through pathways such as *de novo* mutations and epigenetic modifications. For instance, consistent evidence showing increased paternal age to be related to autism (Gardener *et al.* 2009; Hultman *et al.* 2011), led recently to the first whole-genome sequencing study in humans reporting an increase in *de novo* mutations associated with a higher paternal age in autism (Kong *et al.* 2012).

1.4.3 A conceptual framework

Gardener and colleagues recently published systematic reviews of the prenatal, perinatal, and neonatal factors that have been studied in relation to autism (Gardener *et al.* 2009; Gardener *et al.* 2011). They noted a vast heterogeneity in the literature, and commented that small sample sizes, absence of population-based control groups, and retrospective designs based on parental recall could have affected the precision and the validity of several underlying studies. Reporting that over 50 different prenatal factors had been studied, they concluded that some, such as a 'mother born abroad', and taking 'medications during pregnancy' had evidence of an association with a higher risk of offspring autism. These results highlighted the non-specific nature of the evidence, and the need to enhance the understanding of specific exposures and potential pathways through large and methodologically strong population-based studies.

When developing this doctoral study plan on a new data-rich cohort, considering which questions to prioritize in the limited time was a challenge. It was decided to study in detail four questions, each with unique complexities and interpretational challenges, but with the potential to be explained through the relatively underexplored idea of psychosocial stress or adversity during pregnancy in the pathways to autism.

The notion that upsetting a pregnant woman may ‘mark’ her baby is a perception in various cultures. This possibility in the context of autism as an outcome has been discussed for at least three decades (Ward, 1990). A number of reports have been published using data from animal studies reporting that exposure to a variety of stressors during pregnancy may lead to long-term neurodevelopmental and behavioural impairments of the offspring (Glover, 2011; Kinney *et al.* 2008b; Weinstock, 2008). The potential biologically plausible mechanisms cited include alteration in ‘fetal programming’ through epigenetic changes such as via DNA methylation and histone modifications (Schanen, 2006), or through effects on the hypothalamic-pituitary-adrenal axis (Kinney *et al.* 2008b). However, there is relatively little evidence from human studies supporting this theory, since all the studies to date have significant methodological limitations as described later (section 1.4.6).

1.4.4 Parental socioeconomic status

The discipline of social epidemiology is based on the frequently observed findings that many physical and mental morbidities, as well as longevity and mortality follow social gradients. Individuals occupying lower socioeconomic positions (or socioeconomic status, SES) in society are often found to have poorer health related outcomes than their socioeconomically advantaged counterparts (Galobardes *et al.* 2007; Galobardes *et al.* 2008; Honjo, 2004; Krieger *et al.* 1997). Lower measures of parental socioeconomic status are also known to be associated with various child developmental problems (Conger and Donnellan, 2007). However, autism spectrum disorders are a notable exception.

Not unlike the parenting theories, the roots of the associations between high parental socioeconomic status and autism go back to Leo Kanner’s work, and divided the scientific community. Some of Kanner’s followers considered a high socioeconomic status as a prerequisite to an autism diagnosis (Sanua, 1987). Other researchers conceptualized this as a selection bias due to inequalities in access to a diagnosis of autism according to the socioeconomic status of parents (Gillberg *et al.* 1982; Schopler *et al.* 1979a; Tsai *et al.* 1982; Wing, 1980). However, amongst other limitations, these studies were based on very small sample sizes and often had no or inappropriate controls.

Although the interest in this debate subsided as the focus turned to studying the genetics of autism, a systematic search in the preparation of this work (see **Table S1, Paper I**) revealed that the relationship between high SES continued to be reflected in population based literature, particularly in the USA (Bhasin and Schendel, 2007; Croen *et al.* 2002; Durkin *et al.* 2010; Fountain *et al.* 2010; Van Meter *et al.* 2010; Windham *et al.* 2010). Whilst selection factors are cited as a probable explanation, the associations are also often interpreted as being intriguing, baffling and mysterious (Gee, 2010). On the other hand, some studies from countries with universal healthcare provision, found an opposite association (i.e. a lower socioeconomic status linked with higher risk of autism) but these findings received relatively less attention (Dodds *et al.* 2011; Emerson, 2010; Larsson *et al.* 2005). Specifically, the possibility that the social patterning of ASD may be the opposite of Kanner’s original description has not been

considered, although this would be fully consistent with the larger social epidemiology literature on child development.

Further methodologically strong studies on this topic could therefore help understand these contrasting findings; inform the aetiological understanding of autism and also highlight potential social inequalities in access to autism care.

1.4.5 Parental migration

Human beings have migrated from one region to another for various reasons throughout history, but international migration has increased at an unprecedented rate in modern times. Recently, it was estimated that over 200 million individuals are ‘on the move’ globally (McKay *et al.* 2003). The effects of migration on poor mental health are widely documented (Bhugra, 2004). Parental migration has also attracted interest in relation to autism, with Vitamin D deficiency and ethnicity are often suggested as possible explanatory mechanisms (Dealberto, 2011; Keen *et al.* 2010). However, it is also well known that migration in many cases entails exceptionally stressful circumstances and is linked to social disadvantages both in the home country and during resettlement (Bourque *et al.* 2011). In the context of countries like Sweden, this is particularly important because a large proportion of immigration from outside of Europe in recent decades has consisted of asylum granted to individuals fleeing extreme adversities.

The studies on migration and risk of ASD vary markedly in quality, sample sizes and definitions of autism (see systematic review and **Table DS4, Paper II**). They have also produced inconsistent findings, including reports of no associations as well as increased or decreased risks for autism (Barnevik-olsson *et al.* 2010; Croen *et al.* 2002; Gillberg *et al.* 1987; Hultman *et al.* 2002; Kamer *et al.* 2004; Keen *et al.* 2010; Lauritsen *et al.* 2005; Maimburg and Vaeth, 2006; Williams *et al.* 2008b). There is some evidence regarding the possibility of a relationship between migration and autism to be confined to autism with comorbid intellectual disability (Dealberto, 2011), and one study reported a lowered risk for autism with normal or high intelligence (Haglund and Kallen, 2011).

A methodologically sound study with prospective data could help understand the inconsistencies in the literature, and inform the role of the various dimensions of migration in the aetiology of autism. Such a study could also highlight potential health inequalities in migrant health and inform public health action.

1.4.6 Stressful life events

As discussed above in section 1.4.3, the role of prenatal stress in the aetiology of autism has been hypothesised, and has biological plausibility (Kinney *et al.* 2008b). However, robust evidence from human studies is not available. Four previous studies that support the role of stressful events on the risk of ASD included i) an ecological study which found that prenatal exposure to tropical storms was associated with a higher prevalence of ASD (Kinney *et al.* 2008a), but the lack of individual-level data left the results open to a variety of explanations; ii) a small study (n=56) found a higher occurrence of ‘family discord’ reported by mothers of ASD children as compared to controls at

antenatal interviews (Ward, 1990), but its methodology suggested that it was prone to selection, measurement and confounding biases; iii) a study based on maternal recall, found that ASD mothers were more likely to report having stressful life events during pregnancy than controls (Beversdorf *et al.* 2005), but was prone to selection, recall and confounding biases; and iv) a prospective Australian study reported a small but significant association between life-event exposure during pregnancy and autistic traits in 2 year old male, but not female children as measured by a subscale of the Child Behaviour Checklist (Ronald *et al.* 2010). However, the measure of autistic traits was quoted as having modest specificity (42%) as compared with gold standard instruments, and whether this information could be generalised to ASD remained unresolved.

In contrast, the only large population based study using Danish registry data found no evidence for an association between maternal exposure to bereavement during pregnancy and risk of offspring ASD (Li *et al.* 2009), but had a very low cumulative prevalence of ASD and therefore could have been prone to outcome misclassification. The evidence on the role of stressful life events in the development of ASD is therefore not conclusive. However, the biological plausibility of an association warrants further studies on the topic.

1.4.7 Parental depression and maternal antidepressant use

A number of studies have noted associations between parental psychopathology, including depression, and offspring autism. However, two recent meta-analyses could not reach robust conclusions regarding the role of parental depression and ASD (Gardener *et al.* 2009; Yirmiya and Shaked, 2005), citing the absence of methodologically strong studies with prospective data. Since these meta-analyses were published, further studies have reported associations between parental depression and autism but the possibility of reverse causality (Daniels *et al.* 2008), conflating other psychiatric disorders, particularly affective psychoses such as mania with depression (Jokiranta *et al.* 2013), and combining maternal and paternal depression as one exposure limit their utility in a clearer understanding of the specific effects of maternal or paternal depression in associations with ASD (Larsson *et al.* 2005; Lauritsen *et al.* 2005). Furthermore, the relationship between parental depression and autism is often cited as having a genetic origin (Croen *et al.* 2011; Daniels *et al.* 2008). However, an environmental explanation should not be ruled out considering depression has a number of environmental determinants, and genetic mechanisms underpinning it still elusive (Cohen-Woods *et al.* 2013).

A landmark study by Lisa Croen and colleagues, using population-based data on 298 ASD and 1507 control children in Northern California, recently highlighted a potential link between the use of selective serotonin reuptake inhibitor (SSRI) antidepressants during pregnancy and autism (Croen *et al.* 2011). They reported no effect of a maternal depression or other psychiatric diagnosis and autism in the absence of antidepressant use. These conclusions were also supported by several sensitivity analyses. This potential association has biological plausibility (Levitt, 2011), as well as a potential to explain the rising secular trends in ASD prevalence, since SSRI antidepressant use during pregnancy is known to have increased over the past two decades. However, the

numbers were relatively small and the authors urged caution in interpretation and called for further studies on this topic.

Considering the literature for associations between parental depression and ASD is itself inconclusive, it would therefore be important to first clarify whether parental depression is associated with childhood ASD. Longitudinal studies, with measures of depression prior to the birth of the child, comparing the risks of prenatal maternal or paternal depression for offspring ASD may provide insights into whether such an association has a primarily genetic mechanism, or whether potentially reversible environmental influences (such as *in utero* exposure to antidepressants) could be responsible. Also, whether any associations observed for maternal depression can be explained by antidepressant use during pregnancy has implications for advice concerning treatment of depression during pregnancy. Importantly, whether antidepressants other than SSRI's could be considered 'safe' in relation to ASD risk could help inform the pharmacological management of depression during pregnancy, which if untreated is related to substantial risks to mothers as well as their offspring (Spinelli, 2012; Stewart, 2011).

2 AIMS

2.1 OVERALL HYPOTHESIS

The two overarching hypotheses tested in this thesis were:

- 1) That potentially modifiable factors studied during pregnancy or the time preceding birth would be associated with a higher risk of autism in the offspring
- And
- 2) That these risk factors and/or the magnitude of their associations with autism spectrum disorders may be different in autism with and without intellectual disability.

2.2 SPECIFIC AIMS

2.2.1 Study I

The hypothesis for this study was that children belonging to families with a lower socioeconomic status would be at greater risk of ASD in a population-based study with a relatively lower possibility of case ascertainment bias, than studies based in countries without universal healthcare.

2.2.2 Study II

This study aimed to test the hypothesis that parental migration would be associated with a higher risk of offspring autism; and that various dimensions of migration including the geographical region and human development of the country of parental origin, as well as the timing of migration in relation to pregnancy could be important in this context.

2.2.3 Study III

In two population-based studies in Sweden and England with complementary strengths, this study tested the hypothesis that exposure to stressful life events would be associated with an increased risk of offspring ASD, and that the risk would be highest when exposures occurred during the prenatal period.

2.2.4 Study IV

This study examined the hypothesis that parental depression as well as antidepressant use by the mother during pregnancy would be associated with a higher risk of offspring autism.

3 MATERIALS AND METHODS

3.1 RECORD LINKAGE DESIGN

Sweden has a long tradition of maintaining high quality registries recording a wealth of individual-level information which can be linked to individuals and families using unique identifiers. This allows the possibility of record linkage population-based studies, which have made a major contribution to the understanding of human health, morbidity and mortality (Allebeck, 2009). The current thesis is largely based on data from an intergenerational record linkage cohort designed to study the environmental risk factors for autism.

3.2 STUDY POPULATION: THE STOCKHOLM YOUTH COHORT

All of the component projects of this thesis utilize data from the Stockholm Youth Cohort - a register-based, total population cohort of all 589,114 individuals aged 0 through 17 years; who ever lived in Stockholm County between the years 2001 to 2007 (inclusive). Prospective data of these individuals has been linked to their first degree relatives using unique personal identity numbers that are assigned to all Swedish residents (including migrants with a permanent residence permit) (Ludvigsson *et al.* 2009). Specific registers used in the design of the cohort and for the ascertainment of data on exposures, outcome and other characteristics used in this work are described below.

3.2.1 Register linkages in the Stockholm Youth Cohort

3.2.1.1 *The total population register*

The Swedish total population register records, the name, place of residence, sex, date of birth, civil status, place of birth, citizenship, immigration status, and relationships of all Swedish citizens including migrants with permanent residence (Johannesson, 2002; Ludvigsson *et al.* 2009). All individuals on the total population register are assigned a unique personal identity number (PIN) which is also used in other national and regional registers, allowing the possibility of record linkage cohorts. The total population register was used to identify the background population in the Stockholm Youth Cohort.

3.2.1.2 *Multi-generation register*

This register links the PINs of Swedish residents (index persons) with those of their parents and allows the construction of intergenerational cohorts and complex family structures. It is estimated that approximately 97% of mothers and 95% of fathers of index persons who have lived in Sweden since 1961 are linked in this register (Ekbom, 2011). This register was used to identify the parents and siblings of the index persons included in the Stockholm Youth Cohort.

3.2.1.3 The Longitudinal integration database for health insurance and labour market studies (LISA by Swedish acronym)

All individuals 16 years or older, and registered in Sweden on 31 December each year are included in the LISA, which integrates information from social insurance, labour market and education sectors. This register is held and maintained by Statistics Sweden (see http://www.scb.se/Pages/List____257743.aspx) since 1990, and is updated every year. It was used to ascertain information on measures of parental country of birth and socioeconomic status including income, education and occupational class used in this thesis.

3.2.1.4 Medical birth register

The Swedish medical birth register contains information on almost all births in Sweden since 1973, with mandatorily reported data from antenatal, birth and neonatal care. This register was used to abstract contemporaneously recorded information on maternal (including history of diabetes, hypertension, smoking during pregnancy, and prescribed drug use since 1995) (Kallen *et al.* 2011a) and child characteristics (such as Apgar score at 5 minutes, birth weight for gestational age, gestational age at birth) (Axelsson, 2003).

3.2.1.5 National patient register

The National patient register contains the dates and discharge diagnoses of all inpatient (since 1973) and specialist outpatient care (since 2001, but with incomplete psychiatric outpatient data) in Sweden. Diagnoses are coded according to ICD 7-10 (Ludvigsson *et al.* 2011; Swedish National Board of Health and Welfare, 2009).

3.2.1.6 The Swedish cancer register

This register records information on the site, histological type, stage, date and the basis of diagnosis of cancer. Diagnoses are coded according to ICD 7-10 (Barlow *et al.* 2009). Information from this register was used in Study III to identify the diagnosis of cancer in first degree relatives.

3.2.1.7 Cause of death register

This register contains information on the cause of death and the primary and contributory causes listed in the death certificate coded according to ICD 7-10 (Johansson and Westerling, 2000). Information from this register was utilized in Study III to identify deaths in first degree relatives.

3.2.1.8 Stockholm adult psychiatric care register

This regional register records the dates and diagnoses for any contact with specialist outpatient psychiatric services in Stockholm County since 1997. The diagnoses were coded according to DSM-IV groupings until 2004 and according to ICD 10 since 2005 (Jorgensen *et al.* 2010).

3.2.1.9 Pastill: Stockholm child and adolescent psychiatric care register

This regional register records details of child and adolescent psychiatric inpatient and outpatient care within Stockholm County since 2001. The diagnoses are coded according to DSM-IV diagnostic groups until 2008, and according to ICD-10 since 2009 (Idring *et al.* 2012).

3.2.1.10 Stockholm County Council habilitation register

This is a regional register recording details of utilization of the habilitation services provided by the Stockholm County since 1997. The services are provided by separate centres according to type of disability (autism with or without intellectual disability, other intellectual disabilities, mobility, vision or hearing impairments) (Idring *et al.* 2012).

3.2.1.11 Stockholm County Council VAL database

This regional register records public health care services provided by Stockholm County since 1997, including the service provider (clinic) and ICD-10 diagnostic information (available since 2006) (Idring *et al.* 2012).

3.2.2 Autism spectrum disorders in the SYC

3.2.2.1 A brief description of the Swedish system of diagnosis and care of ASD

The public services system in Sweden is well developed, with almost complete coverage, and is used by the overwhelming majority of the population. In Stockholm County, the County Council oversees all ASD related services including healthcare, social care and other services such as special education. All infants and preschool children are invited to well baby clinics where various aspects of child development are assessed. Screening for deficits in developmental domains of social, motor, language and cognitive development take place at specific ages (Hultman *et al.* 2010; Idring *et al.* 2012). Those suspected of autism are referred for a formal assessment. These referrals can also be made by parents, schools or other sources (Axén, 2010).

The diagnostic process involves a multidisciplinary process, with structured assessments being carried out by at least two professionals. The choice of professionals, such as child psychiatrists, neuropaediatricians, clinical or educational psychologists, speech and language therapists, occupational therapist or physiotherapists would be determined by clinical need and presentation. These multi-professional teams are based at neuropaediatric or child and adolescent mental health services (Axén, 2010).

The County Council's guidelines require the diagnostic evaluations to cover a history of the index person's social, medical and developmental history by parental report, observing the individual in their own environment (home, school or nursery), using a structured neuropsychiatric assessment including assessment of cognitive level (standard tests are chosen according to the age and developmental level of the child) (Axén, 2010).

Post diagnostic mental health care is offered by Child and adolescent mental health or child health services. The habilitation services offered by the Stockholm County

Council (http://www.habilitering.nu/gn/opencms/web/HAB/andra_sprak/english) provide and coordinate other follow-up care such as rehabilitation, special education, occupational therapy, and social care. In Stockholm County, the habilitation services for autism are organized into two types of centres, which accept children with ASD based on the presence or absence of a comorbid intellectual disability.

3.2.2.2 Registers used to identify ASD in the Stockholm Youth Cohort

Data from the regional Stockholm Child and Adolescent Psychiatric Care Register, the habilitation register, and the VAL register and the National Patient Register were used to identify individuals with autism (Idring *et al.* 2012).

3.2.2.3 Validity of ASD cases

Two validation procedures were conducted and are described fully elsewhere (Idring *et al.* 2012).

Case note validation:

The case note validation was conducted on a random selection of 200 cases of ASD from the sample (100 cases of ASD with ID and 100 ASD without ID). A child and adolescent psychiatrist and a resident in neuropaediatrics independently reviewed 177 case-notes using a proforma based on current diagnostic criteria. An ASD diagnosis was supported in 170 (96%) of the 177 case-notes that could be retrieved and studied.

Cross validation with a national twin sample:

Data from a national population-based study of twins (the Child and Adolescent Twin study in Sweden-CATSS) were used to cross validate the sample of twins in the SYC [27 twins with ASD, 2721 without ASD] that was also assessed in CATSS (Lichtenstein *et al.* 2010). The twins were screened for ASD based on an assessment for neurodevelopmental disorders, the A-TAC (Larson *et al.* 2010) by telephonic interview (Lichtenstein *et al.* 2010). Twenty three (85.2%, 95% CI 66.2–95.8) of 27 ASD individuals in SYC screened positive for ASD in the CATSS, and less than 1% (95% CI 0.7–1.4) of the non-case twins in the SYC screened positive for ASD diagnosis in CATSS.

3.2.3 Nested case-control design

For all the studies, each case of autism spectrum disorder was matched by birth date (month and year) to 10 randomly selected controls from within the Stockholm Youth Cohort, who were alive and free of autism at the time of case ascertainment. Common exclusion criteria included children who were adopted or those who had been living in Stockholm County for less than four years (thus also excluding all children aged less than 4 years).

3.2.4 Exposure variables

3.2.4.1 Socioeconomic Status (SES)

The Longitudinal Integration Database for Health Insurance and Labor Market Studies (<http://www.scb.se>) was used to collect prospectively recorded information on the socioeconomic variables. The data collected were from the year before birth of the index child, or closest available.

It has been widely reported that socioeconomic status is a multidimensional concept (Galobardes *et al.* 2007), and therefore three measures of socioeconomic status were used- income, education and occupational class.

Individualized disposable family income:

This measure reflects the income of a household after accounting for all sources of income (including social benefits) for a family, after deduction of all outgoing expenses (including taxes) and adjusted for family size. This information is based on mandatory reporting and is updated yearly by Statistics Sweden. To avoid inflationary bias due to the change in the value of money over time, the information recorded at the time of the child's birth was extracted and coded into quintiles, in relation to the year of ascertainment.

Parental education:

This information was available as years of completed education for each parent. Since the interest in this variable was as a measure of *family* socioeconomic status, apart from studying each parent's education level separately, a variable depicting the highest educational achievement of the mother or father was also created. These variables were studied categorically, comprising education up to 9 years (primary school), 10-12 years (secondary school) and over 12 years (higher education or university).

Occupational class:

As for parental education, the parental occupational class of each parent was studied separately but also as a grouped variable of the highest of either mother or father using six categories based on Swedish guidelines (Statistics Sweden, 1982). These include: unskilled manual worker, skilled manual worker, lower level non-manual employee, intermediate level non-manual employee, high level non-manual employee and self-employed. A seventh 'unclassified' category was created to include students, homemakers and other economically inactive groups in the analysis for parents with no occupational class defined in the register. This information was extracted from records of the most recent Census in Sweden in 1990 and therefore it was not possible to time it to the year of birth for all index children.

3.2.4.2 Maternal migration

The mother's country of birth was used to define migration status and studied using two distinct groupings aimed to capture two distinct dimensions of the parental region of origin that may be important in relation to the development or identification of offspring autism.

The first grouping was done by coding the countries into geographical region of birth (United Nations definitions, <http://unstats.un.org/unsd/methods/m49/m49regin.htm>). The second grouping was created using an estimate of human development of the country of origin (using the UNDP Human Development Index, a composite indicator of development derived using indicators of life expectancy, education and income <http://hdr.undp.org/en/statistics/indices/hdi>). A third dimension, timing of the migration (in years, before or after) in relation to the birth of the child was also studied.

3.2.4.3 Serious Life events:

The records of all first degree relatives of the index population were linked with the Swedish National Cause of Death Register, The Swedish Cancer Registry, and The National Patient register. These registers were used to identify deaths, serious accidents, injuries or events; and a diagnosis of life threatening or serious illnesses in the first degree relatives using ICD-8, 9 and 10 codes (**Table 3.2.1**), and then timed in relation to the birth of the child- in the year before pregnancy, during pregnancy and during 1, 2 and 3 completed years after birth.

Table 3.2.1. Diagnostic categories for identifying events occurring in first degree relatives

	ICD10 code
Death of a parent and sibling	
Death caused by suicide (including undetermined intent)	X60-X84, Y10-Y34
Death caused by accident	
Death by all other causes	
Serious illness of a parent and sibling	
Meningitis	A39, B004, G00-G01, G04-G05
Myocardial infarction, cardiac arrest	I21-I22, I46
Pulmonary embolism	I260, I269, O88
Cerebral infarction, cerebral hemorrhage, stroke (NOS)	I60-I64, O873, O225
Malignant neoplasms	C00-C75, C81-C97
Serious injury/accident of a parent and sibling	
Intracranial injury, fracture of skull and facial bones , Crushing injuries to head and face	S020-S021, S023-S024 S027-S029, S064-S069, S070-S079
Burns and corrosions of respiratory tract, other internal organs, and of >49% of body surface	T27-T28, T293, T297, T315-T319, T325-T329
Asphyxiation and other accidental threats to breathing	T71, W75-W84
Anaphylactic shock, certain reactions to transfusion, infusion, therapeutic injection	T780, T782, T802, T805, T809
Traumatic shock	T794, T811
Complications of trauma or transfusion, infusion, therapeutic injection; air embolism, fat embolism	T790, T791, T800
Accidental drowning and submersion	W65-W74, T751
Assault, maltreatment syndromes	X85-Y099, T74
Legal intervention and operations of war	Y35-Y36
Shock from lightning or electric current	T750, T754, X33
Poisoning and toxic effects	T36-T65, X40-49
Injury of internal organs and blood vessels	S15, S25-S28, S35-S37
Event of undetermined intent*	Y10-Y34
Suicide attempt	
Suicide attempts**(intentional self-harm and event of undetermined intent)	X60-X84, Y10-Y34

Note: This table, along with complete ICD- 8 and 9 codes can be found in full at:
Prenatal and Early Life Exposure to Stressful Life Events and Risk of Autism Spectrum Disorders: Population-Based Studies in Sweden and England. Table_S1.doc. Rai D et al; PLOS ONE. 10.1371/journal.pone.0038893.s001.

3.2.4.4 Parental history of depression and antidepressant use

The psychiatric history of parents was identified using the Stockholm County Adult Psychiatric Outpatient Register and the Swedish National Patient Register. Mothers and fathers with depression were considered to have a prenatal history of depression if they had a registered diagnosis of a depressive episode, recurrent depressive disorder, persistent mood disorder, other or unspecified mood disorder before the birth of the study child. Other diagnoses were grouped into psychotic disorders (including

schizophrenia and other non-affective psychoses, and affective psychoses such as bipolar disorder), neurodevelopmental or personality disorders, alcohol and drug disorders, anxiety, somatoform and other disorders.

In case parents had more than one listed diagnosis, two different approaches were used. First, a hierarchy based on the ICD-10 (World Health Organization, 1993) was developed keeping in mind the research question. A parent was considered to have the highest diagnosis from the list below in case they had more than one recorded diagnosis:

- Schizophrenia/non-affective psychoses or bipolar disorder,
- Neurodevelopmental or personality disorders
- Alcohol and drug disorders
- Depression
- Anxiety disorders
- Somatoform disorders or any other disorder not listed above

Table 3.2.2 International classification of diseases diagnostic codes used to identify a history of depression and other psychiatric disorders in parents before the birth of the index child.

Diagnosis group	ICD10 (1997-)
Alcohol/drugs misuse	F10-F19
Schizophrenia	F20-29
Bipolar disorder	F30-31
Depression	F32-39
Anxiety disorders	F40-43
Somatoform disorders	F44-48
Personality disorder	F60-F69
Neurodevelopmental disorders	F70-F82, F84, F889, F90, F95, F98 5,6

Therefore, in the above approach, if there were multiple diagnoses, depression was only considered a priority over anxiety or somatoform disorders. Specifically, individuals with a history of psychotic disorders, neurodevelopmental or personality disorders and drug and alcohol disorders were not counted as having depression in this strategy. In the second strategy, individuals were allowed to fall in the different diagnostic groups if more than one diagnosis was identified (**Table 3.2.2**).

Maternal antidepressant use during pregnancy

Since 1995, data on medication use reported at the first antenatal interview are abstracted in the Medical Birth Register (Kallen *et al.* 2011a). The data are collected by midwives and then using a semi-automated system, coded into the register using the Anatomical Therapeutic Classification (ATC) of drugs produced by the World Health Organisation (http://www.whocc.no/atc_ddd_index/).

Therefore, for children born from 1995 onwards, data on any antidepressant use (ATC code N06A) were retrieved. These were further divided into the two most commonly used antidepressant classes- selective serotonin reuptake inhibitors (SSRI, ATC N06AB), and non-selective monoamine reuptake inhibitors (non-selective MRIs, ATC code N06AA).

3.3 THE AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN (STUDY III)

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a large birth cohort study that was established to explore the environmental, social, psychological and genetic factors associated with child health and development (Golding *et al.* 2001). Unlike the methodology of other birth cohort studies before it, ALSPAC recruited the index children not at birth, but when their mothers were pregnant, and captured an extensive amount of data prospectively. Pregnant women in the Bristol area of England who had an expected date of delivery between April 1991 and December 1992 were recruited. A total of 14,541 pregnant women were recruited, resulting in 14,062 live births (Golding *et al.* 2001). Prospective information from expectant mothers (and their partners) during the prenatal period and the time of birth was collected. The study, currently in its 22nd year, has continued to collect information from the mothers, children and other sources at regular intervals which include questionnaires, clinical measures and biological samples (Boyd *et al.* 2012; Fraser *et al.* 2012).

3.3.1 Autism spectrum disorders in ALSPAC

Children diagnosed with ASD were identified through National Health Records and the Pupil Level Annual Schools Census (PLASC) (Williams *et al.* 2008a). These diagnoses were validated in relation to ICD-10 criteria by a team led by an experienced consultant paediatrician (Williams *et al.* 2008a). A total of 86 children with an ASD diagnosis were identified in ALSPAC by age 11.

3.3.2 Life event measures in ALSPAC

In ALSPAC, data on over 40 different common and rare life events were collected at multiple times in pregnancy and childhood, with items based on known inventories (Barnett *et al.* 1983; Brown and Harris, 1978; Honnor *et al.* 1994). For the present study, 6 time points were included. These included data collected from questionnaires administered at 18 weeks gestation (covering events from beginning of pregnancy), 8 weeks postnatal (covering events from mid-pregnancy), 8 months postnatal (covering events since the birth of the child), 21-months postnatal (covering events since the child was 8 months old), 33-months postnatal (covering events since the child was one and a

half years old), and at 3 yr 11 months postnatal (covering events since the child was two and a half years old).

The life event questions included a measure of perceived impact of each life event. Each life event, at each time point was coded 0 if the event did not occur, 1 if event occurred but mother reported not being affected by event, 2 if event occurred and mother reported being minimally affected by it, 3 if event occurred and mother reporting being moderately affected by it, and 4 if event occurred and mother reported being severely affected by it.

Weighted life events scores for each of the 6 time points studied were derived by adding the above values for each of the life events studied. The weighted life event scores were also categorized into a binary variable into two categories depicting the top quartiles, and the lower three quartiles of the scores. Separate variables were also made which included the total number of life events, irrespective of their perceived impact.

3.4 STATISTICAL ANALYSIS

The analyses in Papers I and II were conducted using SAS software version 9.2 for Windows (SAS Institute Inc., 2008), and those in Papers III and IV using Stata version 10.1 for Windows (StataCorp., 2007).

In each of the studies, the association between the defined exposures and the autism outcomes was studied using multivariable regression methods, allowing for control of a number of potential confounders. In all studies, a number of sensitivity analyses were conducted to ensure robustness of the findings and to rule out other explanations. Full details of the analysis of each study are available in the individual publications appended with this thesis (Magnusson *et al.* 2012; Rai *et al.* 2012a; Rai *et al.* 2012b; Rai *et al.* 2013).

The statistical methods used in the studies included:

Conditional logistic regression: was the main analytic method used to study the associations between the exposures and the outcomes using the Stockholm Youth Cohort data in Studies I to IV. This method allows the estimation of odds ratios and their 95% confidence intervals for the respective associations while accounting for the matching of the cases and controls by age and sex, and allowing additional adjustment of potential confounding variables (Kirkwood and Sterne, 2003).

Polychoric correlation: to estimate the relationship between the various measures of socioeconomic status [Study I].

Cochrane Armitage Trend Test: to calculate p-values for trend [Studies I and II].

The X^2 test: to calculate p-values for association between categorical variables in ALSPAC [Study III].

Unconditional logistic regression: to calculate odds ratios and 95% CI's for autism spectrum disorder with exposure to life events in ALSPAC [Study III].

The **X² test of heterogeneity**: to calculate $p_{\text{heterogeneity}}$ values to assess the statistical significance of any differences between estimates for paternal and maternal depression, and for ASD with and without ID [Study IV].

Population attributable fractions (PAFs): were calculated to estimate the proportion of autism cases that could be prevented if antidepressant use were completely eliminated from the population, assuming the association was causal and all confounders had been accounted for [Study IV].

3.5 ETHICAL APPROVAL

The Research Ethics Committee at Karolinska Institutet, Stockholm, provided ethical approval for the record linkages and studies based within the Stockholm Youth Cohort. The use of data from the Avon Longitudinal Study of Parents and Children was approved by the ALSPAC Executive committee, and originally collected after approval by the ALSPAC Ethics and Law Committee (see <http://www.bristol.ac.uk/alspac/researchers/data-access/ethics/>).

4 RESULTS

4.1 STUDY I

A total of 4709 children with ASD and 46489 age and gender matched controls with complete data were available. These included 2744 cases of ASD without ID, and 1965 cases of ASD with ID.

The main findings were that:

- Children with ASD were more likely to come from families with a lower individualized household income (p-value for trend <0.001) (see **Fig 4.1.1**)
- Parental manual occupations, particularly unskilled manual work were more likely to be associated with an increased risk of offspring ASD (see **Fig 4.1.2**)
- There were no important relationships observed between parental education and ASD (see **Fig 4.1.3**)
- The associations did not change materially after adjustment for any of the potential confounders, including ages of both parents at birth of the child, migration status, parity, and history of psychiatric service use in the mother and father
- These relationships followed a similar pattern when ASD cases were categorized into those with or without co-morbid intellectual disability (see **Paper I - Table 2**).

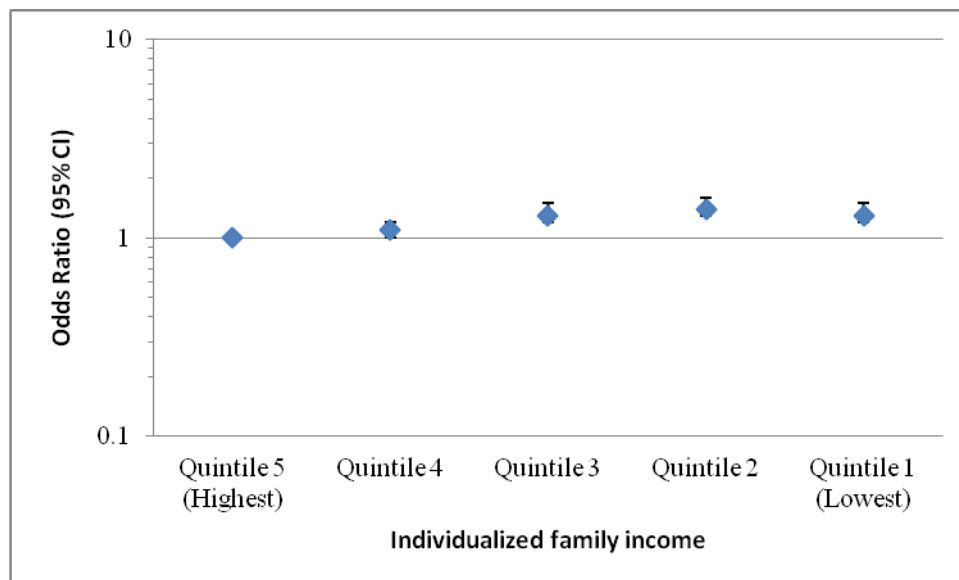


Fig 4.1.1 Odds ratios depicting associations between individualized household income and offspring autism spectrum disorder (adjusted for parental education and occupational class, and for father's and mother's age at child's birth, migration status (both parents born in Sweden, 1 parent born in Sweden, both parents born abroad), parity, and parental psychiatric service use (yes/no for each parent))

In additional analyses similar results were found after

- introducing other characteristics, including maternal report of smoking at the first antenatal visit, birth weight for gestational age, gestational age, and the infant's Apgar score at 5 minutes
- including only the first ASD case in each family (ASD n=4584)

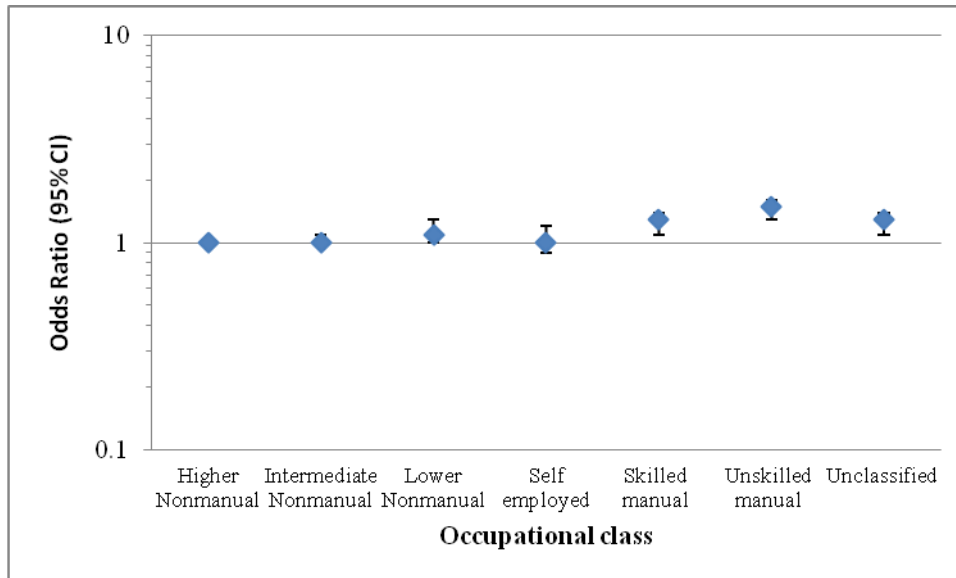


Fig 4.1.2 Odds ratios depicting associations between parental occupational class and offspring autism spectrum disorder (adjusted for parental education and individualized household income, and for father's and mother's age at child's birth, migration status (both parents born in Sweden, 1 parent born in Sweden, both parents born abroad), parity, and parental psychiatric service use (yes/no for each parent))

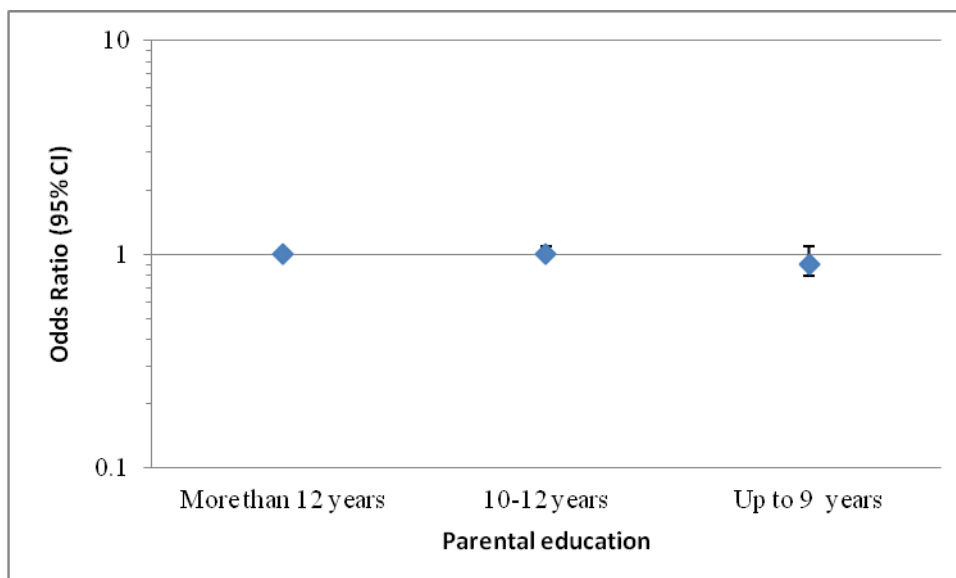


Fig 4.1.3 Odds ratios depicting associations between parental education and offspring autism spectrum disorder (adjusted for individualized household income and occupational class, and for father's and mother's age at child's birth, migration status (both parents born in Sweden, 1 parent born in Sweden, both parents born abroad), parity, and parental psychiatric service use (yes/no for each parent))

4.2 STUDY II

The study population for this paper comprised 3918 children with ASD (n=2269 for ASD without ID and n=1649 for ASD with ID) and up to 10 age and sex matched controls. Three dimensions of migration were studied which included the geographic region of birth of the mother, the human development index of the country of birth and the timing of migration in relation to pregnancy.

The associations between the geographic region of birth of mothers and offspring ASD followed clearly opposite relationships depending upon intellectual disability. There was almost a 50% increase in odds of ASD with ID in children of migrant parents, as compared with children of parents born in Sweden (crude OR 1.5, 95% CI 1.3-1.7). This relationship varied with the region of maternal birth. In contrast, quite the opposite associations were observed for ASD without ID. Children of migrant parents had markedly reduced odds of ASD without ID (crude OR 0.5, 95% CI 0.5-0.6). This relationship was observed in almost every migrant group, including those in which no elevated associations with ASD with ID were found. (See **Table 4.2.1**)

TABLE 4.2.1. Associations between maternal region of birth and offspring autism spectrum disorders (ASD), and ASD with and without Intellectual disability

	All ASD	ASD without ID	ASD with ID
Maternal country of origin	Adjusted OR 95% CI	Adjusted OR 95% CI	Adjusted OR 95% CI
Northern Africa	1.0 (0.7-1.4)	0.3 (0.2-0.7)	1.5 (0.9-2.4)
Eastern Africa	1.1 (0.9-1.4)	0.3 (0.2-0.5)	1.9 (1.5-2.5)
Other African	1.5 (1.0-2.2)	0.2 (0.0-0.7)	3.5 (2.5-5.6)
Northern America	N/A	N/A	N/A
Latin America/ Caribbean	1.1 (0.9-1.4)	0.5 (0.4-0.8)	1.6 (1.2-2.2)
Southern Asia	0.8 (0.6-1.0)	0.3 (0.2-0.5)	1.3 (0.9-1.7)
Western Asia	0.6 (0.5-0.7)	0.3 (0.2-0.4)	0.9 (0.7-1.2)
Other Asian	0.9 (0.6-1.3)	0.6 (0.4-1.1)	1.1 (0.6-2.0)
Northern Europe	1.1 (0.9-1.3)	1.1 (0.8-1.4)	0.9 (0.6-1.2)
Eastern Europe	0.9 (0.7-1.3)	0.8 (0.6-1.2)	0.8 (0.5-1.3)
Southern Europe	0.6 (0.4-0.9)	0.3 (0.2-0.5)	1.1 (0.8-1.7)
Western Europe	1.0 (0.6-1.7)	0.8 (0.4-1.7)	1.0 (0.4-2.2)

Adjusted OR: Adjusted odds ratio for maternal and paternal age at child's birth and family disposable income at child's birth or in early life, as applicable. Countries contributing the largest number of cases in each sub-region were (when applicable): Northern Africa – Morocco; Eastern Africa – Somalia and Ethiopia; Latin America and the Caribbean – Chile; Southern Asia – Iran; Western Asia – Iraq and Turkey; Northern Europe – Finland; Eastern Europe – Poland; Southern Europe – Former Yugoslavia

To study a different dimension of migration, the maternal country of origin was coded based upon the UNDP Human Development Index (HDI). A decreasing HDI was associated with elevated odds of ASD with ID, and the opposite was observed for ASD without ID (see **Fig 4.2.1**).

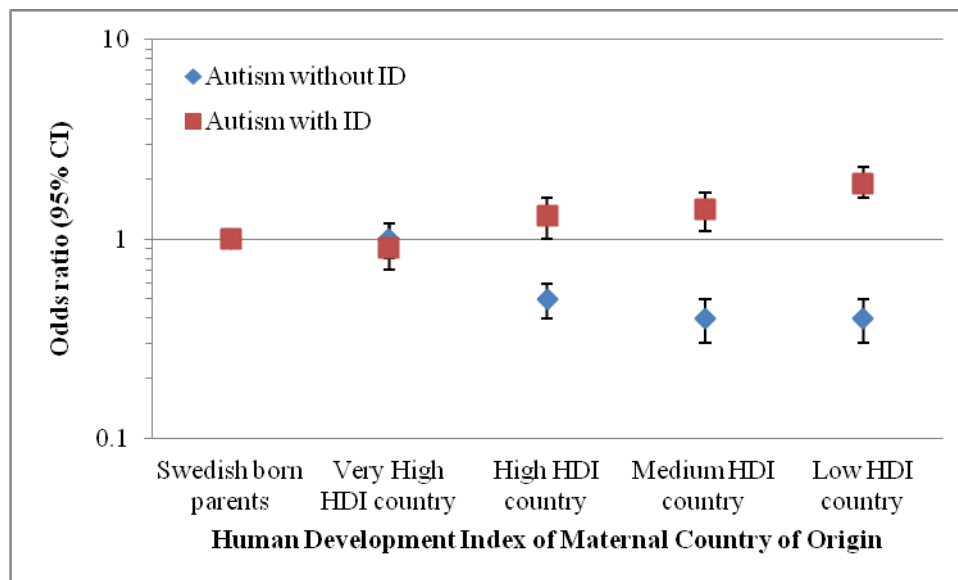
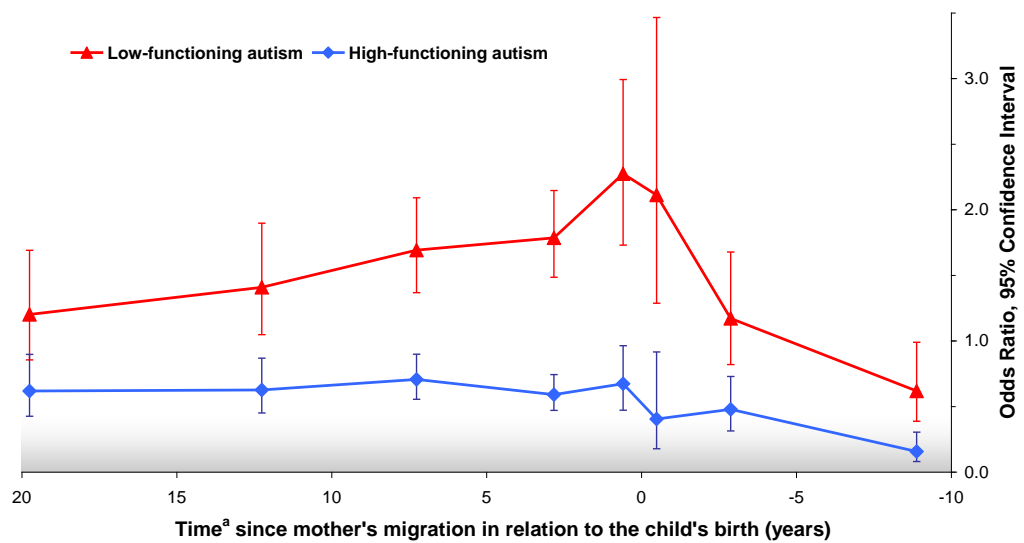


Fig 4.2.1 Odds Ratios depicting the relationship between the Human Development Index of Maternal Country of Birth and offspring ASD with and without Intellectual Disability. Adjusted for maternal and paternal age at child's birth and family disposable income.

Finally, the timing of maternal migration in relation to the birth of the index child was studied. These analyses showed a non-linear pattern in the relationship between ASD with ID with the highest odds observed for mothers who had migrated within the year before the birth of the child, used as a proxy for migration during pregnancy (crude OR 2.3, 95% CI 1.7-3.0). Such a pattern was not observed for ASD without ID (See **Fig. 4.2.2**).

Additional analyses mutually adjusting the timing and the geographic region of maternal migration amongst children with both parents born abroad found both of these characteristics to have independent associations with ASD with ID (See **Paper II, Table 4**).

The results did not substantially change after adjusting for parental ages, and income, a characteristic highly correlated with migration from low income countries (See **Paper II, Tables 1-4**). Additionally, adjusting for obstetric complications (in an analysis restricted to children born in Sweden, since obstetric data were only available for children born in Sweden) did not explain the associations.



^aPositive values indicate time since maternal migration to Sweden before the child's birth (in 2nd generation immigrant children), negative values indicate age of child when the mother migrated to Sweden (in 1st generation immigrant children).

Fig 4.2.2 Odds Ratio's of ASD with and without intellectual disability (marked low and high functioning autism respectively) in children with both parents born abroad as compared to those with both parents born in Sweden, by time since maternal immigration in relation to the child's birth. This figure is based on the original figure published in Paper II, and reproduced with permission from the publisher.

4.3 STUDY III

Results from the Stockholm Youth Cohort:

For this analysis, 4429 children with ASD (n=1828 for ASD with ID, n=2601 for ASD without ID) and 43277 controls with complete data were available. The severe life events occurred in less than 1% at all time points and were proportionately similar in case and control mothers during the prenatal period (**Table 4.3.1**).

Table 4.3.1: Proportion of mothers of ASD cases and controls experiencing a serious life event in the Stockholm Youth Cohort

Timing of Stressful life event	Cases n=4429 (%)	Controls n=43277 (%)	p*
Year before pregnancy	0.93	0.73	0.135
During pregnancy	0.38	0.38	0.992
1st year after birth	0.65	0.53	0.242
2nd year after birth	0.68	0.58	0.391
3rd year after birth	0.86	0.57	0.012

*p-values derived from conditional logistic regression to account for matching

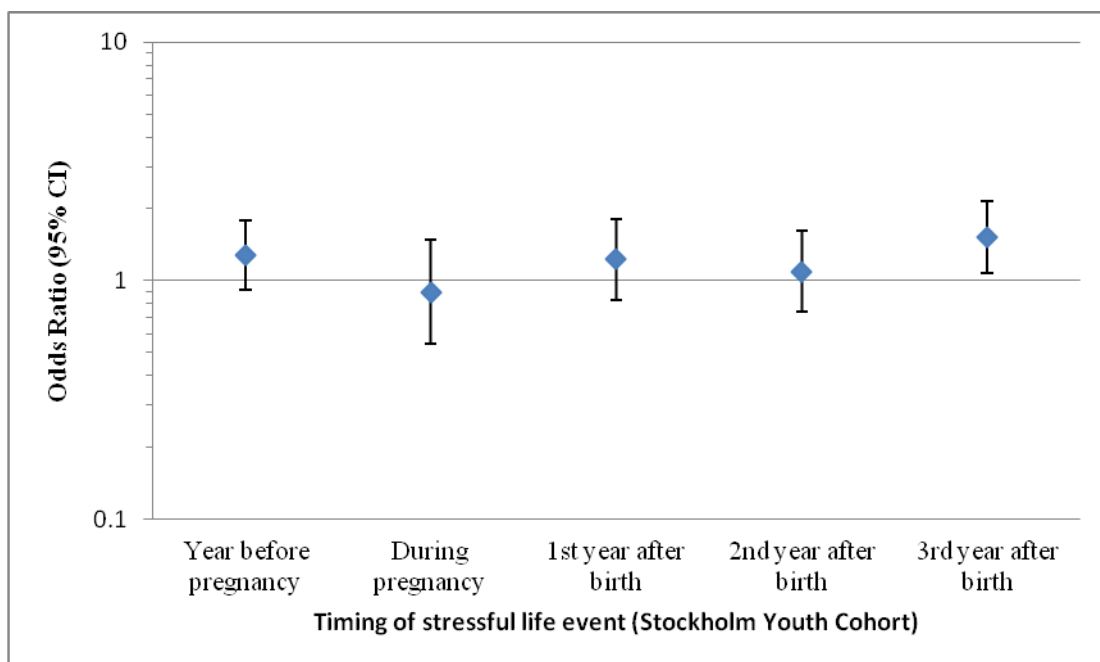


Fig. 4.3.1 *Conditional logistic regression models estimating association of the mother's experience of a serious life event and risk of offspring ASD in the Stockholm Youth Cohort, adjusted for age of mother and father at birth of child, parity, quintiles of family disposable income adjusted for family size, highest educational qualification of mother or father, occupational class (highest of mother or father) and migration status of parents.*

In conditional logistic regression analysis, no evidence of a relationship between life events and ASD was found in either crude or adjusted conditional logistic regression analyses but the confidence intervals were wide, probably reflecting the small numbers (See **Fig. 4.3.1**).

Similar results were found when ASDs were grouped by intellectual disability. However, the point estimates of risk when life events occurred during pregnancy were higher for ASD without ID [OR 1.21 95% CI (0.67-2.20)] than those for ASD with ID [OR 0.47 95% CI (0.17-1.31), See **Paper III, Table 3**].

Results from the Avon Longitudinal Study of Parents and Children:

The response rate to the life events questionnaire varied in ALSPAC, from 9616 mothers in the 3 year 11 months questionnaire to 11554 mothers in the 8 week postnatal questionnaire (see **Table 4.3.2**). Mothers who were in the top quartile of weighted life event scores at baseline were more likely to be non-responders in all subsequent time points studied after the 8 week postnatal questionnaire (χ^2 20.270, d.f=1, $p<0.001$) but were no more likely to have a child subsequently diagnosed with ASD, which was assessed by clinical records and record linkage (χ^2 0.650, d.f=1, $p=0.420$).

Table 4.3.2 Descriptive statistics of life event exposures in mothers of children with and without ASD in ALSPAC

Timing of life event	N (ASD/ no ASD)	Percentage in top quartile of weighted life event score (%)		
		Mothers of children with ASD	Mothers of children without ASD	p*
Early Pregnancy to 18 weeks	73/11153	19.2	22.6	0.489
Mid Pregnancy to 2 months post delivery	70/11484	20.0	22.4	0.628
Birth to 8 months	72/11177	13.9	22.5	0.081
8 to 21 months	69/10311	15.9	25.0	0.084
18 to 33 months	65/9655	13.9	22.9	0.082
2.5 years to 3 years 11 months	64/9552	14.1	23.1	0.088
*P-values derived from Chi-square test				

The exposure variable studied in ALSPAC included both rare and common life events (See **Paper III, Table S2**), and the majority of the mothers at any time point reported having experienced one or more of the life events in the questionnaires.

There was no evidence for an association between prenatal weighted life events score (incorporating the number and severity of life events) and a diagnosis of ASD in the ALSPAC data in either crude or adjusted logistic regression models (see **Fig 4.3.2**).

When the exposure variable was divided into the top quartile of the weighted life events score versus the bottom 3 quartiles, similar results were found (See **Paper III, Table 6**), but the associations between life events during the prenatal period and ASD appeared to increase when life events at other time points were simultaneously included in the regression model [Odds ratio for exposure in early pregnancy OR 1.25 95% CI (0.54-2.89), and for late pregnancy OR 1.45 95% (0.61-3.44)].

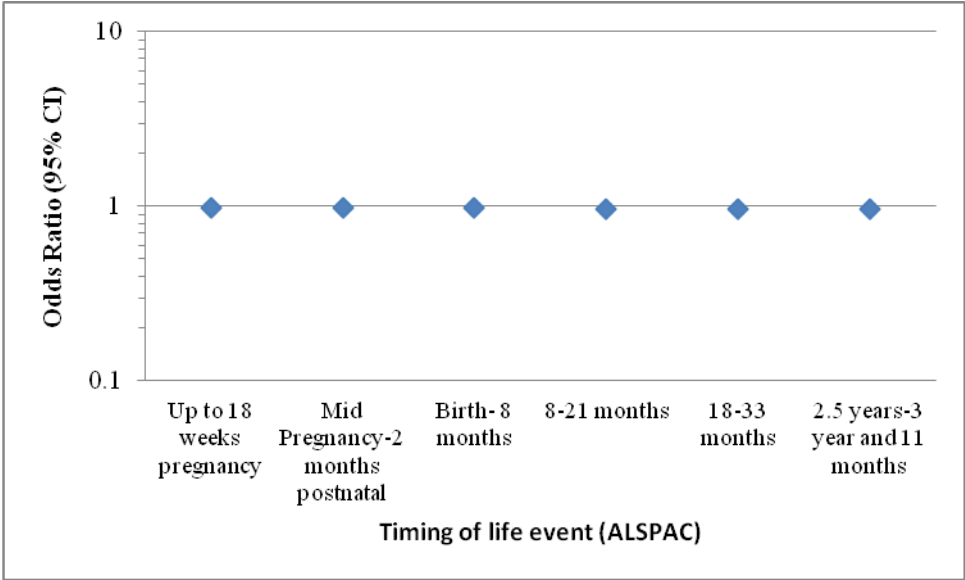


Fig. 4.3.2 Results of logistic regression analysis to study the association of maternal weighted life event scores (as a continuous variable) and offspring ASD at the time points studied in ALSPAC. The model is adjusted for maternal and paternal age, occupational class (highest or either parent), educational qualification (highest of either parent), tenure of accommodation, parity and sex of child.

4.4 STUDY IV

Parental depression and ASD

There were 4429 cases (1828 with and 2601 without a recorded ID) and 43277 controls with complete data in the main analysis.

Maternal depression was associated with an approximately 60% increase in risk of ASD [Crude OR 1.61 95% CI (1.17-2.23), $p=0.004$], which was not substantially attenuated after adjustment for all potential confounders. When the analysis was conducted separately for ASD with and without ID, the increased risk of ASD with maternal depression appeared to be largely related to an almost two fold odds of ASD without ID, and there was no evidence for an increased odds of ASD with ID (See **Fig 4.4.1**). The odds ratio for ASD with and without ID were statistically heterogeneous ($p_{\text{heterogeneity}}=0.04$).

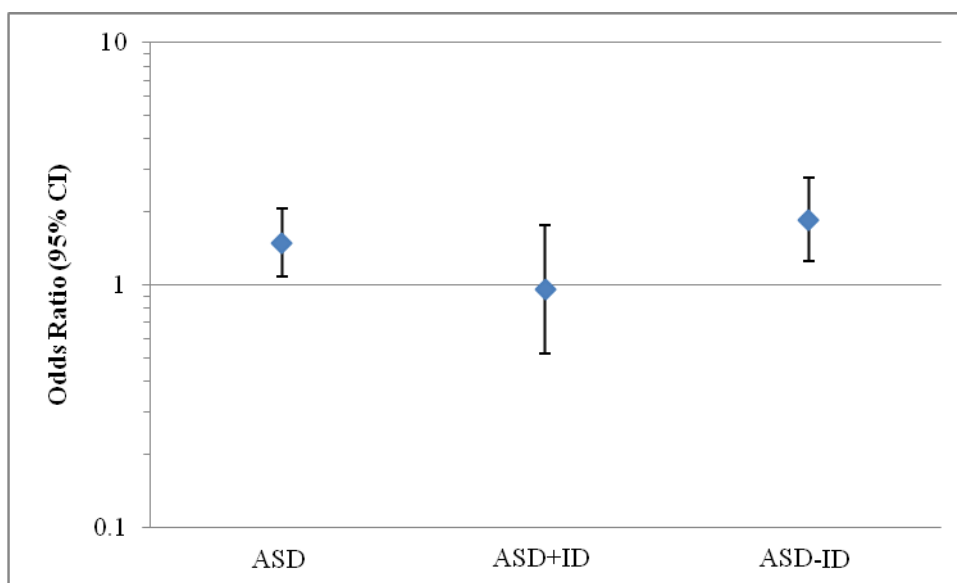


Fig 4.4.1 Conditional logistic regression analysis to estimate the associations between a prenatal history of maternal depression and offspring ASD, ASD with (ASD+ID), and without intellectual disability (ASD-ID). The model is adjusted for the age of parents, household income, parental education, parental occupational class, parental migration status, and parity, any other psychiatric condition in the mother and depression or any other psychiatric condition in the father.

No evidence for an association between depression in fathers and offspring ASD was found, and this was the case for both ASD with and without ID (See **Fig. 4.4.2**), but the difference between the results for maternal and paternal depression were not statistically different ($p_{\text{heterogeneity}}=0.71$).

A similar pattern of associations was observed in a number of sensitivity analyses (See **Paper IV - Fig 1 and Tables S3 to S6**), as well as when, in a smaller sample the regression models were further adjusted for birth weight for gestational age, gestational age at birth, Apgar score at 5 minutes, maternal smoking, and recorded maternal diabetes or hypertension (**Paper IV - Table S7**).

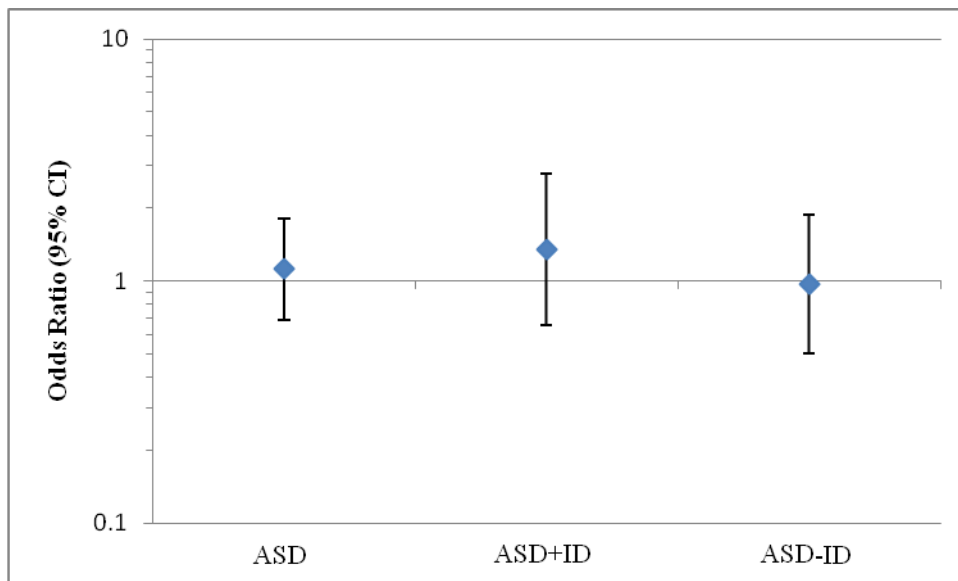


Fig 4.4.2 Conditional logistic regression analysis to estimate the associations between a prenatal history of paternal depression and offspring ASD, ASD with (ASD+ID), and without intellectual disability (ASD-ID). The model is adjusted for the age of parents, household income, parental education, parental occupational class, parental migration status, and parity, any other psychiatric condition in the father and depression or any other psychiatric condition in the mother.

Maternal depression, antidepressant use during pregnancy and ASD

Information for medication use during pregnancy was available for 1679 ASD cases (743 ASD cases with, and 936 ASD cases without ID) and 16845 control children who were born from 1995 onwards. The associations of maternal depression and ASD in this sample followed a similar trend, as the main analysis above, but with wider confidence intervals. These associations were attenuated upon adjustment for maternal antidepressant use during pregnancy (see **Paper IV – Table 2**).

The children of mothers with a history of depression who also reported antidepressant use during pregnancy appeared to have strong associations with ASD, as compared with those without depression and with no antidepressant use. These associations were also found in ASD without ID but not in ASD with ID. Maternal depression without antidepressant use during pregnancy was not associated with a heightened risk of ASD (See **Fig. 4.4.3**).

There was an almost two- fold increase in risk of ASD with antidepressant use during pregnancy (Adjusted OR 1.90, 95% CI 1.15-3.14). This association was observed for ASD without ID, but not for ASD with ID. Maternal use of both SSRI and non selective MRI antidepressants were associated with higher risks, although there were few individuals in these analyses and the confidence intervals were wide (See **Paper IV - Table 4**).

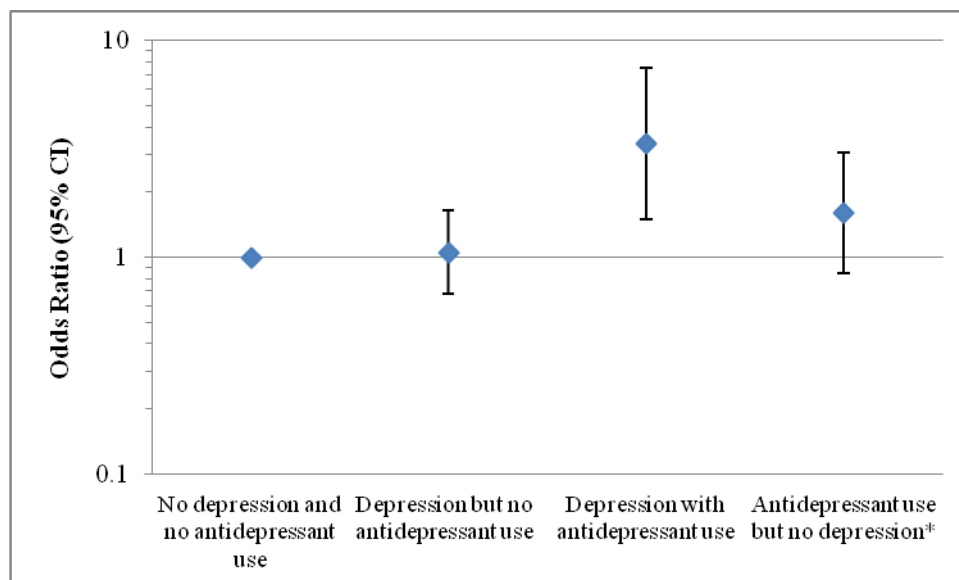


Fig 4.4.3 *Conditional logistic regression analysis to estimate the associations between a variable combining a prenatal history of maternal depression with antidepressant use during pregnancy, and offspring ASD. The model is adjusted for the age of parents, household income, parental education, parental occupational class, parental migration status, parity, and a history of any other psychiatric condition in the mother.*

Assuming the association between antidepressant use during pregnancy and autism spectrum disorders was causal, and that all confounding had been accounted for, the population attributable fraction estimate suggested that a complete elimination of all antidepressant use during pregnancy would lead to 0.6% of ASD to be prevented.

5 DISCUSSION

5.1 MAIN FINDINGS IN THE CONTEXT OF PREVIOUS LITERATURE

5.1.1 Socioeconomic patterning of autism may be similar to other developmental disorders

Measures of a lower socioeconomic status in families – specifically, lower household income, and unskilled, manual or unclassified occupations were associated with a higher risk of autism in this population-based study in Sweden.

The associations were typically modest. However, they were in an opposite direction to those described historically, and to several contemporary reports from the USA showing relatively strong relationships with measures of higher socioeconomic status (Bhasin and Schendel, 2007; Croen *et al.* 2002; Durkin *et al.* 2010; Fountain *et al.* 2010; Van Meter *et al.* 2010; Windham *et al.* 2010). The associations were consistent with results of studies from Denmark and Canada (Dodds *et al.* 2011; Larsson *et al.* 2005), which like Sweden, are countries where universal healthcare is available.

The associations were also consistent with a larger body of research suggesting links between a lower parental socioeconomic status and several cognitive and developmental outcomes in children including inattention, speech impairment, poor executive function, and delays in cognitive and socio-emotional development (Bradley and Corwyn, 2002; Hackman and Farah, 2009; Hoff, 2003; Mezzacappa, 2004). In the present study, the relationships were similar for ASD with and without intellectual disability, and therefore it is unlikely that the associations were driven by the known association of lower socioeconomic status with intellectual disability.

5.1.2 Migration and autism- relationships beyond geographic conceptualizations

In Study II, maternal migration had divergent relationships with ASD with and without intellectual disability- showing heightened risks for ASD with ID and reduced ones for ASD without ID. Importantly, this study found that associations of migration with ASD not only varied by the geographical region of origin of parents, but also by the human development of the region of origin, as well as the timing of migration in relation to pregnancy.

The findings of differential relationships of parental migration between ASD with and without intellectual disability are supported by a smaller study from the Malmö region of Sweden (Haglund and Kallen, 2011), and since the publication of Paper II, have been replicated in a population based study in the Netherlands (Van Der Ven *et al.* 2012). These findings may also explain the inconsistency of the relationship between parental migration and autism observed in other population based studies (Croen *et al.* 2002; Hultman *et al.* 2002; Lauritsen *et al.* 2005; Maimburg and Vaeth, 2006; Williams *et al.* 2008b) since the studies had varying definitions of autism as well as the

respective proportions of children with ID (see **Table DS4 in supplement to Paper II**).

5.1.3 No evidence of a link between acute life events and autism

In Study III, no evidence for a relationship between stressful life events during pregnancy and a heightened risk of autism was found in two cohorts with data on severe and common life events during the perinatal period, along with measures of perceived severity in one cohort.

The findings were consistent with a recent study using data collected from Danish Registries which reported no significant relationships between the experience of bereavement by mothers during pregnancy and risk of offspring ASD (Li *et al.* 2009). The results however, contradicted some positive associations reported between prenatal stressful events and ASD, although the studies reporting these had significant methodological limitations including an ecological design (Kinney *et al.* 2008a), and a high probability of selection and recall biases (Beversdorf *et al.* 2005; Ward, 1990).

5.1.4 Depression or antidepressant use during pregnancy?

In Study IV, a higher risk of autism was associated with a prenatal history of maternal depression, but did not appear to be associated with paternal depression. These associations were higher for ASD without ID, and were not observed for ASD with ID. In a smaller sample, when maternal antidepressant use was simultaneously studied, the associations of maternal depression with autism appeared to be limited to the group of women who reported taking antidepressants during pregnancy.

This study extends the findings of the only other study simultaneously studying maternal depression and antidepressant use during pregnancy in relation to offspring autism (Croen *et al.* 2011). Using a larger sample, it highlights that the associations appear to be specific to ASD without intellectual disability, and that the results were also apparent for use of antidepressants other than SSRI's, which also have serotonergic activity. However, confounding by maternal depression as an indication is still a possibility (discussed in methodological considerations) and these results require caution in their interpretation.

5.2 METHODOLOGICAL CONSIDERATIONS

5.2.1 Record linkage designs in epidemiological studies

Record linkage designs such as the current one based within the Stockholm Youth Cohort allow the possibility of conducting intergenerational studies on entire populations with little loss of follow-up. Such studies would otherwise be prohibitively costly and require extensive resources to conduct using other designs requiring questionnaire data or clinical measurements. Record linkage designs are a particularly efficient way of studying uncommon disorders such as autism where only a small proportion of the entire population would develop the disorder. However, the success of such studies entirely depends upon the quality and completeness of underlying records which are used in the linkages. As described in *Methods*, Sweden has a long tradition of maintaining high quality registries capturing an extensive amount of contemporaneous

data on a various facets of health, disease and socioeconomic factors, which are well maintained and regularly updated making the conduct of high quality record linkage studies possible.

5.2.2 Selection bias

Selection bias could lead to misleading conclusions if the relationship between the exposures and the outcome is different for the cases included in the study and all the eligible cases in the population (Rothman *et al.* 2009). It is less likely in the context of the Stockholm Youth Cohort, where the majority of the diagnosed ASD cases in the county were likely to have been captured by the registers and thus included in the studies. Furthermore, selection of controls in the studies was done at random from a matched sample (by age and sex) to the entire background population who were free of an autism diagnosis at the point of ascertainment, making any control selection bias unlikely.

Selection bias may also occur due to loss to follow up, or missing data in any of the variables which may lead to exclusion of certain cases or controls from analysis. Most of the registries that the Stockholm Youth Cohort is based on have relatively little missing data. One of the unavoidable reasons for missing data in these studies were when children were born abroad, and therefore the strategy of sensitivity analyses including children born in Sweden only was employed. Further, parental migration was included as a covariate in each study.

For Study III, it has been established that mothers with socioeconomic disadvantages were more likely to drop out over time in the ALSPAC cohort (Wolke *et al.* 2009), and therefore selection bias due to a loss to follow up may be a possibility. In further examination of this issue, it was found that mothers with higher weighted life-events (exposure) were less likely to respond to further questionnaires after the initial two time points under study, but non-response was not associated with a future diagnosis of ASD in the child (outcome). This was reassuring since it has previously been shown that attrition based upon exposure characteristics usually does not result in biased estimates, but that attrition based upon outcome can (Wolke *et al.* 2009). Several socioeconomic characteristics that are known to be associated with attrition in ALSPAC were included as covariates to further minimize any possibility of such bias (Wolke *et al.* 2009).

5.2.3 Information bias

This type of bias may arise due to misclassification or measurement error and could relate to exposures and outcomes (as well as covariates) and may be differential or non-differential.

5.2.3.1 Outcome misclassification

The multisource ascertainment of cases using various sources apart from health service use, in combination with the wide coverage of child development screening services in Sweden is likely to have ensured that the majority of diagnosed autism cases in Stockholm County were identified. However, there is a possibility that undiagnosed

cases continue to remain in the community and in its strictest sense, it would be reasonable to consider the outcomes in this thesis as ‘detected autism’.

Since ASD diagnoses are made by multi-professional teams, according to comprehensive guidelines, systematic biases in the diagnostic labelling based on any of the exposure characteristics are less likely. The validation studies conducted indicated that the case ascertainment had high sensitivity as well as specificity (Idring *et al.* 2012). Furthermore, children less than 4 years old were not included in the analyses since diagnoses below this age may be less reliable. This censoring was achieved by removing all individuals living in Stockholm County for less than 4 years- a strategy that additionally allowed a reasonable period of time for migrant families to be captured in the registries.

The inability to classify ASD cases according to current DSM-IV or ICD-10 subcategories can be considered a limitation since these were not recorded consistently across the registers. However, both these diagnostic systems are currently under revision, and the strategy of studying ASD as an overall group are fully consistent with proposals for future classification, since the boundaries between the individual subcategories have not been supported in the literature (American Psychiatric Association, 2011; Lord *et al.* 2011).

Furthermore, the alternative strategy used to study two categories of ASD based on co-morbid intellectual disability is supported by empirical evidence from clinical, epidemiological and genetic studies (Bartak and Rutter, 1976; Szatmari *et al.* 2007; Wing, 1981), and reflects service delivery models for autism in Sweden, UK and many other parts of the world.

5.2.3.2 Exposure measurement and misclassification

Measurement error and exposure misclassification should be considered for some of the variables in the individual studies. None of these are likely to be differential for cases and controls and therefore it is not likely that the associations would have been biased. However, the possibility of such misclassification should be acknowledged. The availability of prospectively collection information on exposures, before the child was born would have minimized the possibility of recall bias.

In Study I, occupational class was only available from the most recent Swedish census in 1990. Individuals such as students, home-makers and other economically inactive groups do not have occupational classes in these data, and were termed ‘unclassified’. There is a possibility that some of these individuals such as young students at the 1990 census went on to have an occupational class later but were not correctly classified.

In Study II, the mother’s country of birth was used to characterise parental migration, but the paternal and maternal countries of birth were largely identical in children with both parents born abroad.

In Study III, the SYC only had record linkages with details of the offspring’s first degree relatives and therefore, it was not possible to study the exposures in relation to

events occurring in other relatives of the mother- therefore reflecting an under-ascertainment of the life events. However the use of ALSPAC data in parallel showing a similar lack of associations was reassuring.

In Study IV, depression was identified using secondary care records and therefore under-ascertainment was certain, since most individuals with depressive disorders do not seek help or are managed in primary care, a problem that is common to other population-based studies on this topic. The antidepressant data in Study IV were abstracted from maternal reports at the first antenatal interview conducted at a median time of approximately 10 weeks gestation, and possibly provides a better estimate of actual use as compared with data extracted from prescription records. Previous studies have shown that the Medical Birth Register identified 78% of all antidepressants prescribed during the first trimester, (Stephansson *et al.* 2011) and the drug name registered in prescription records and that recorded in the MBR had a 97% concordance (Kallen *et al.* 2011b; Stephansson *et al.* 2011).

5.2.4 Confounding

Confounding may occur when the relationship between exposure and outcome is affected by factors which are associated with the exposure, and also independently associated with the outcome. For instance, a confounder could be a common cause of both the exposure and the outcome. Confounding may result in the observed estimates between the exposure and the outcome to be biased in either direction. It is one of the major sources of bias in observational studies.

In the present studies, a range of potential confounders were assessed and controlled for using multivariable regression methods, but the possibility of residual confounding can never be ruled out. In particular, despite efforts to control for psychiatric diagnoses, the presence of autism or its related traits in the parents could not be fully captured and therefore confounding by genetic factors in the observed associations remains a possibility. Adjustment for downstream exposures that could be potential mediating characteristics was done separately to avoid collider-stratification bias (Janszky *et al.* 2010).

Confounding by indication was a particular concern in Study IV, where it was not possible with the current design and data available, to robustly assess whether the relationship between antidepressant use during pregnancy and ASD reflected confounding by severe depression during pregnancy.

5.2.5 Statistical power

Although the studies were large, the exposures in Studies III (SYC) and IV, and the outcome in Study III (ALSPAC) comprised relatively small numbers, leading to wide confidence intervals, and therefore limited precision of the estimates. Specifically for Study III, a lack of evidence for an association due to limited statistical power cannot be excluded because of these wide confidence intervals.

5.2.6 External validity

The studies were population based and included all recorded cases of ASD in Stockholm County during the time period studied and therefore the findings are readily generalisable to the Swedish population. They also represent some of the largest studies conducted on the individual topics studied and are likely to have good external validity to other European countries and possibly beyond.

5.3 POSSIBLE EXPLANATORY MECHANISMS

Causal inferences based on observational epidemiological data should be made with caution, and the methodological limitations above must be kept in mind when interpreting the results. Taken together, the studies in this thesis highlight relationships observed for autism spectrum disorders with prenatal measures of parental socioeconomic status, various aspects of maternal migration including geographical region and timing, and a history of depression and antidepressant use during pregnancy. Two out of the four studies (Study II and IV) found marked differences between the associations of the exposures and autism spectrum disorder with and without intellectual disability. However, since the heightened risks in study II and IV were observed for ASD with and without ID respectively, maternal stress exposure as an overarching explanatory mechanism for *all the* associations found is unlikely.

5.3.1 Study I

Had the outcome not been autism, the parental socioeconomic gradients observed in Study I would be unsurprising since these findings are shared by several developmental problems. Such a social patterning is often explained using two theories, social causation and social selection (Conger and Donnellan, 2007; Dohrenwend *et al.* 1992). The findings in the context of the social causation hypothesis would suggest that stresses associated with a lower parental socioeconomic status may impact negatively on child development and eventually lead to the development or expression of ASD. The social selection hypothesis, in contrast would convey that rearing a child with developmental problems such as ASD may lead to a downward social mobility in the parents who may have to give up employment or take up employment with lower incomes. The design of the present study in which the socioeconomic status of parents was measured at the time of birth of the child, with additional analyses including only eldest children allowed this as an explanatory pathway responsible to be ruled out.

The difference of the findings from historical descriptions and recent US studies is interesting. The origins of the theory that a high parental SES was linked with ASD came from the founding father of child psychiatry, Leo Kanner. He first described autism to the English speaking world and also authored the first textbook of child psychiatry (Kanner, 1972), which continued to feature these views till the 1980s (Sanua, 1990) and may have influenced the views of clinicians and researchers. It has been noted that some clinicians and early studies considered a high socioeconomic status as one of the diagnostic features of autism (Schopler *et al.* 1979b). This was further highlighted by a study by Cucarro *et al.*, which reported a systematic difference in professional's preference of the label of 'autism' based on parental socioeconomic status (Cuccaro *et al.* 1996). In contrast, European scholars of autism propagated the

view that Kanner's views reflected a selection bias related to access to autism diagnosis, using population based approaches which albeit led to very small number of cases (Gillberg *et al.* 1982; Wing, 1980).

The discrepancy between the results of this study with other contemporary reports from the USA may therefore be explained by this historical basis of conceptualizing the social patterning of autism, combined with a greater equality in access to care in the Swedish system. The findings of the present study therefore support the hypothesis that the socioeconomic patterning of autism may be similar to other developmental disorders once biases in case ascertainment are minimized.

5.3.2 Study II

The differences observed between parental migration and the risk of ASD with and without intellectual disability were intriguing. Intuitively, a misclassification of these groups due to possible biases in the diagnostic procedures related to language barriers, cultural misunderstanding, prejudice or differential properties of diagnostic instruments could be hypothesised. However, the evidence behind this explanation was weak since, the reduced risks of ASD without ID were observed for almost all geographical regions, irrespective of whether a corresponding increase in the risk of ASD with ID was observed or not. Also, the finding of a variable risk of ASD with ID dependent upon the time of migration in relation to pregnancy with no such trend observed for ASD without ID goes against the possibility of the results being simply due to misclassification of outcome. Furthermore, such differences between migration and the risks of ASD with and without ID have now been reported by two other studies, in Sweden and the Netherlands respectively (Haglund and Kallen, 2011; Van Der Ven *et al.* 2012).

It is impossible to rule out discrete etiological mechanisms leading to a true protective effect of migration on ASD without ID, but an under-diagnosis in migrant children may also be an explanation. It is possible that the relatively nuanced social deficits related to ASD without ID in migrant populations could be attributed to cultural differences and therefore missed, while intellectual disabilities are more readily identifiable. It is also possible that a low perceived need for mental health care, stigma and lower awareness of service availability in migrant populations (Bradby *et al.* 2007; Fassaert *et al.* 2009), may all result in reduced help-seeking in the absence of clear and problematic developmental delays (Begeer *et al.* 2009). Furthermore, there is also very little known about the cross-cultural validity of instruments used in the diagnosis of autism.

There are several possible reasons for the finding that ASD with intellectual disability was over-represented in migrant families. One possible explanation is that the underlying ASD rates vary in different countries of origin. However, not much information on the prevalence of ASD in low and middle income countries is available (Fombonne, 2003). A variation of prevalence in relation to ethnicity or skin colour (for instance, a darker skin colour is considered to increase the risk of vitamin D deficiency, a potential risk factor for autism) (Dealberto, 2011) is also possible but not consistent with risks changing in relation to timing of migration. Also, an independent effect of

ethnicity on offspring autism was not found in the absence of migration in a recent UK study (Keen *et al.* 2010), and studies from the USA found both elevated (Bhasin and Schendel, 2007; Croen *et al.* 2002) and reduced (Centers for Disease Control and Prevention, 2012) risks of autism in non-Hispanic Black and Hispanic children compared to White children.

As discussed previously, other aspects of migration such as stressful circumstances in the process of migration, and social disadvantage in the country of origin as well as while settling in Sweden are often overlooked. The analysis coding migration according to the human development of the maternal country of birth found increasing risks of ASD with ID as the levels of human development decreased. The finding of a varying association of ASD with ID with the timing of maternal migration, with risks appearing to peak when migration occurred in the year before birth also indicate that potentially modifiable factors, particularly during pregnancy could be at play in the aetiology of ASD with intellectual disability.

5.3.3 Study III

There are several explanations for the ‘null’ findings between life events and ASD in the two cohorts. Firstly, the null hypothesis may be true. In other words, there may be no association between maternal life event related stress during pregnancy and autism. Secondly, as discussed in methodological considerations, there is the possibility that there was insufficient statistical power to find a marginal association in both the cohorts used, as well as in a recent Danish study (Li *et al.* 2009). It may also be possible that acute stressors such as life events do not have sustained effects and therefore less relevant to foetal neurodevelopment than other potential chronic stressors such as clinical depression or adversities related to lower socioeconomic status or migration. Finally, it may also be possible that life events related stress have differential relationships with some component traits of autism as was reported in a recent Australian study (Ronald *et al.* 2010).

5.3.4 Study IV

In the larger cohort, a diagnosis of maternal but not paternal depression appeared to be associated with ASD, and this relationship appeared to be largely driven by associations for ASD without ID. Although an association of ASD with depression in fathers could not be ruled out due to relatively wide confidence intervals, the findings suggest that the association between parental depression and offspring ASD cannot be assumed to be similar for both parents. Any conceptualisation of the genetic basis of such associations should also be able to explain the above inconsistency, and it should be remembered that a substantial proportion of the variance in depression in adults is attributable to environmental factors (Cohen-Woods *et al.* 2013).

An environmental pathway should therefore not be ruled out in the relationship between maternal depression and ASD. In a subsample, there was evidence that the use of selective serotonin reuptake inhibitors, as well as other monoamine reuptake inhibitors (which act on serotonergic as well as other monoamine pathways) (Boyer and Shannon, 2005) during pregnancy was associated with a higher risk of ASD; and

children of mothers who had a history of depression but no reported antidepressant use did not appear to have this higher risk.

However, it is important to note that antidepressant use may simply be a proxy for severe and active depression during pregnancy. Therefore, the possibility of confounding by indication impossible to rule out, and further work will be required to disentangle these associations. The consistent differences observed for associations between ASD with and without ID in relation to maternal depression and antidepressant use highlight further that studying them separately might be informative in relation to the aetiology of autism.

6 CONCLUSIONS AND FUTURE DIRECTIONS

Using a large population-based sample in Sweden with prospective data, the aim of this work was to study in detail prenatal exposures that, if causal in relation to autism, may act via environmental pathways. In three of the four studies there was evidence of a relationship between the prenatal factors studied and autism. In two studies, the timing of the event (migration, antidepressant use or severe depression during pregnancy) was indicative of pregnancy related exposures, but resulted in a higher risk of autism with *and* without intellectual disability respectively, highlighting the value of studying autism with and without intellectual disability separately. However, in light of these inconsistencies, a single explanatory mechanism such as the effect of prenatal stress is unlikely to explain all of the associations observed.

The findings highlight several issues and raise new questions for future research.

The findings of opposite socioeconomic gradients in autism in Sweden as compared to contemporary studies in other countries, particularly the United States of America warrant further scrutiny. If these represent inequalities in access to care, it would be important to understand how they could be tackled. For instance, studies investigating diagnostic biases based on health-care professional's perceptions such as the one by Cucarro *et al.* (Cuccaro *et al.* 1996) could be repeated to understand contemporary perceptions regarding the diagnostic labelling of autism.

Social epidemiologists have long argued that different socioeconomic measures may be important according to the varying socio-cultural contexts, and multiple measures are recommended in epidemiological studies to minimize confounding due to socioeconomic status (Conger and Donnellan, 2007; Galobardes *et al.* 2007; Krieger *et al.* 1997; Oakes and Rossi, 2003). In the preparation of this work, it was noted that many epidemiological studies of autism did not include any socioeconomic measures, or include a single measure such as maternal education (Bilder, 2009; Glasson, 2004; Hultman *et al.* 2002; Lauritsen *et al.* 2005; Maimburg and Vaeth, 2006; Williams *et al.* 2008b). In light of the present results, it might be important to study multiple measures of socioeconomic status, since they might have varying and independent relationships with ASD.

Study II highlights the need for further scrutinising and replicating the findings of timing of migration in relation to pregnancy. A better understanding of the cross-cultural validity of the diagnostic processes used for autism ascertainment is also warranted. In particular, it would be important for future Swedish research on the topic of migration and autism to investigate whether the seemingly protective effect of migration on autism without intellectual disability is related to cultural biases in diagnostic labelling, or differences in help seeking in migrants. It would be important to investigate whether the relationship between migration and autism with intellectual disability is unique to autism, or whether it is common to all intellectual disabilities.

The population-based studies to date on the association between life events during pregnancy and risk of offspring ASD have not been able to rule out an association due to wide confidence intervals. This question could be studied in larger cohorts, as they become available.

Further work will also be required to disentangle the role of maternal depression versus antidepressant use during pregnancy in the pathways to autism. It is likely that data from other large cohorts will be available in the coming years to allow meta-analyses and subgroup analyses for individual medications potentially implicated. Until then, caution would be required before assuming any associations are causal, and it would be important to acknowledge that if causal, only a small minority of ASD cases would be prevented by hypothetically eliminating antidepressant use during pregnancy. If this issue arises within clinical discussions, the possibility that the associations may reflect the risk of autism related to the severest forms of depression during pregnancy would need to be acknowledged, and informed decisions made after helping patients weigh the risks of untreated depression (Spinelli, 2012; Stewart, 2011) with all known adverse outcomes related to antidepressant use during pregnancy. (Lund *et al.* 2009; Oberlander *et al.* 2006; Occhiogrosso *et al.* 2012; Rahimi *et al.* 2006; Reis and Kallen, 2010; Stewart, 2011)

As the knowledge of the aetiology of autism grows, it will be important to carefully examine the possible mediators of the observed relationships. Complementary designs such as sibling-control analyses and studies with both genetic and environmental data may allow for greater control for potential genetic confounding.

In conclusion, the results of this thesis suggest that studying environmental risk factors for autism may be a goal worth pursuing, and could inform the understanding of the pathways to autism. Future studies should attempt to detail the relationships between other candidate environmental factors and autism spectrum disorders. Where possible, autism spectrum disorders with and without intellectual disabilities should be studied separately since these categories may have different determinants.

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